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PRUNE DWARF

E. M. HILDEBRAND

(Accepted for publication January 17, 1942)

INTRODUCTION

Since the first announcement in 1936 (7), important information has been accumulated on the dwarf disease of prune (Prunus virus 6 (6); *Nanus pruni* H (5)). Originally found only in Niagara County, New York, the disease has since been observed in Canada. From the economic standpoint it is a minor disease, which, for some unknown reason, has become locally a serious problem in a few orchards. The standard control recommendation for the prompt removal of diseased trees has not eliminated the trouble, since in certain cases it has reappeared again within a few years. While studies aimed at solving this difficulty have not yet been concluded sufficient progress has been made during the past 6 years to deserve publication.

DAMSON PLUM MASKS PRUNE-DWARF VIRUS

The causal virus of prune dwarf may be even more widespread than is realized at present owing to the fact that the Damson plum has been found to harbor the virus without showing symptoms. The discovery that the prune-dwarf virus was harbored by Damson plum came about as the result of top-working a few Damson misplants to Italian prune in one of the commercial orchards under observation. In 1932 this orchard of about 230 trees had 2 widely separated diseased trees immediately adjacent to, respectively, 7 and 3 erect growing plums, identified as Damson, that had accidentally gotten into the orchard. When the cions that grew following grafting in 1933 all showed foliage symptoms of prune dwarf two possibilities were suggested: (1) that the grower had inadvertently used cions from diseased trees or (2) that the Damson plum trees harbored the causal virus.

All attempts to demonstrate the presence of the virus in the Damson plum trees in the orchard mentioned have given positive results. Cions and buds from Damson and from both healthy and diseased Italian prune were taken from this orchard and indexed on Italian prune trees at Ithaca in 1935, 1937, and again in 1939, employing a minimum of two trees for testing each source, with the same results each time. Transmission of the dwarf disease was always effected from the symptomless Damson trees and the diseased Italian prunes but never from symptomless trees of Italian prune.

VIRUS HARBORED IN NURSERY AND ORCHARD DAMSON PLUMS

To check on how widespread were virus carriers among Damson plums, trees were procured from several sources including nurseries and orchards, 3 from within and 2 from without New York State. Not all sources contained the virus but 1 out-of-state source and 2 from within New York gave

positive results when indexed on Italian prune. How widespread this virus may be in plum varieties that mask symptoms remains to be determined, but, thus far, indications are that still other varieties may be involved.

In 1939 G. H. Berkeley and R. S. Willison showed the writer an interesting case near Grimsby, Ontario, where a grower had topworked Damson plums to Italian prunes about 3 years previously, with the result that a large percentage of the prune cions became infected. This observation confirmed the results of the New York experiments, and the evidence at hand rather conclusively demonstrates the fact that some of the Damson plums are masked carriers of prune-dwarf virus.

VIRUS SYMPTOMS MASKED BY BRADSHAW PLUM

That the Bradshaw plum might be a masked carrier of the prune-dwarf virus was indicated when symptoms failed to develop following bud inoculation of trees, but proof of this fact has only recently been established. In one Italian prune orchard from which all diseased trees had been previously eradicated a new outbreak occurred in 1936, followed by a further increase in the number of diseased trees in 1940. Careful examination revealed two Bradshaw (Niagara) plum trees amidst the newly infected ones. Dormant scions taken from the trees in question were grafted on two plants each of several varieties of plums including Italian (Fellenberg), Lombard, Damson, and Bradshaw. Symptoms developed within 6 weeks on Italian prune and Lombard plum. Whereas the Damson and Bradshaw varieties remained normal in appearance, it was subsequently demonstrated by indexing on peach seedlings, employing a rapid subsequently technique (4), that they were harboring the virus.

RATE AND MANNER OF SPREAD IN ORCHARDS

Evidence on rate and manner of spread of prune-dwarf virus between trees in orchards over a 10-year period may provide a clue as to the natural agent of dissemination. In the orchards under observation, which varied each year from 3 to 7 during the above period, the increase in number of diseased trees was intermittent. Although most of the orchards were removed before yielding data on this point, two exceptions will be cited. Orchard A contained 58 trees, approximately 25 per cent of which were diseased in 1932. The grower removed the defective ones and replanted to prunes. The disease reappeared on one tree in 1937. The number of diseased trees jumped to 6 in 1940, and, although they were hit and miss, they were confined to the same end of the orchard. Orchard B, about 230 trees, showed 2 diseased trees in 1932, 11 in 1937, 13 in 1938, and 16 in 1940, when the entire orchard was removed. The new infections all appeared immediately adjacent to the two initial infections. Both orchards had severe infestations with the green plum aphis in 1936 and lighter infestations in 1939.

The possibility of spread by the pruning operation seems remote, since

the common mechanical transmission methods that have been tested have always failed to give positive results.

The above circumstantial evidence that the green plum aphis may be the vector of prune dwarf has not been borne out thus far by caging experiments with this insect (7). However, after concluding the above mentioned caging experiments, one of the 4 prune trees developed symptoms in 1935. The fact that this tree was in the open and exposed to environmental factors beyond control makes the evidence merely suggestive, not conclusive. Besides, some other agent than the aphid may have been involved.

The tendency for this disease to spread to the immediately adjacent healthy trees, with occasional skips, suggests a short flight range of the natural vector, a possibility that fits in rather well with the suspicion that an aphid may be involved. The studies on insect transmission, conducted both in the greenhouse and outdoors, that have been in progress for several years have not been concluded.

EFFECT ON YIELDS

The striking effect of this disease on fruitfulness can be gleaned from actual yields of young prune and plum trees shortly after they came into bearing. Two adjacent 6-year-old Italian prune trees, one diseased and the other healthy, yielded as follows: (1) total number of fruits, 7 and 115; total weight of fruit, 8 and 101 ounces; average weight of fruit, 1.14 and 0.88 ounces. The yield from the diseased tree was less than 8 per cent of normal and closely approximates the estimates on yields of old orchard trees, which have ranged between 5 and 10 per cent of normal over a period of years. Whereas the prune type of plum has two striking and distinctive symptoms of dwarfing and narrowing of leaves and almost complete loss of fruitfulness due to abortion of pistils, the effect on Lombard plum (Fig. 1) seems to be largely confined to leaf symptoms. The fruit yield of a healthy Lombard plum tree compared with the average yield of 3 adjacent diseased trees was as follows: (1) number of fruits, 115 and 175; (2) total weight of fruits, 85 and 97 oz.; (3) average weight of fruit, 0.74 and 0.55 oz. The smaller size of fruit on the diseased trees was attributed to the larger number of fruits and to the reduced leaf surface. The fruit yields of symptomless Damson plum trees that carried the virus seemed not to be affected at all.

TRANSMISSION EXPERIMENTS

Although the original experiments (7) indicated that only plums were susceptible to infection by the prune-dwarf virus, additional experiments have been conducted on plums, cherries, and peaches.

The customary inoculation procedure was to place a diseased bud or cion at least midway up the stem of a young 1- or 2-year-old plant, and, where 2 buds or cions were used, at least one was placed high on the stem.

Plum to Plum

The only essential for successful transmission from prune to prune was

tissue union between cion and stock for a relatively short interval of a few weeks. Several types of graft (bud, cleft, whip) employed in 8 separate experiments, involving 5 or more trees each, all proved equally effective and 100 per cent efficient in transmission experiments with the prune. The

FIG. 1. Lombard plum tree, severely affected with prune dwarf, on which the foliage is dwarfed but the fruit crop apparently normal. The inset shows portion of Italian prune tree with characteristic dwarfing of foliage and sparsity of fruit.

details of these experiments have been omitted to save space. It was found that placing the grafts midway or higher up on the stem hastened the appearance of symptoms. The incubation period varied from about 5 weeks

to a year or longer, depending on when during the year the inoculations were made, the position of the grafts on the stems, and the growth condition of the plants employed.

The first symptoms to appear on prune were chlorotic rings (Fig. 2) on otherwise normal appearing leaves in close proximity to the position of the diseased tissue about 5 weeks after grafting. As the season advanced this symptom had a tendency to become less conspicuous, whereas the later-formed leaves in the tip region became markedly dwarfed. Figure 2 illus-

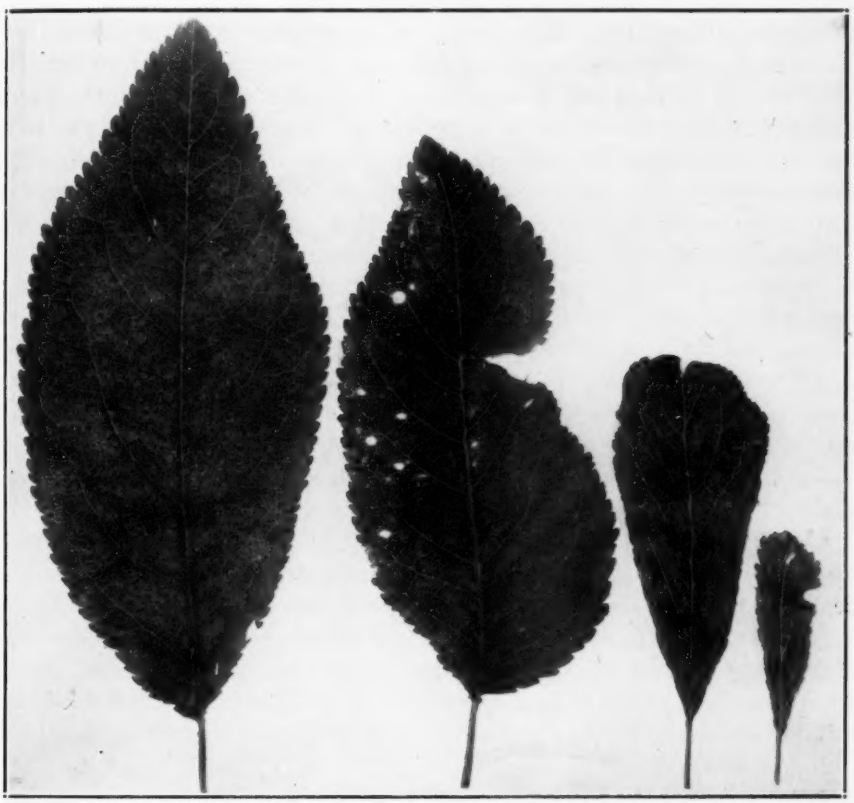

FIG. 2. Symptoms of dwarf on current-season foliage of Italian prune—ring spot, shot hole, malformation, and dwarfing—representing the range in expression from the base to the tip of a single shoot of a tree, in September, that had received a diseased bud early in the spring.

trates typical leaf symptoms on a plant in September, which had been budded at the beginning of the growing season, ranging from chlorotic rings near the base to the dwarfed narrow leaves at the tip of a shoot.

Transmission experiments conducted between Italian prune and several plum varieties: (*Prunus domestica*—Italian (Fellenberg), Smith, German, Lombard, Bradshaw and Reine Claude; *P. salicina*—Abundance, Burbank, and Red June; *P. insititia*—Damson (Shropshire)) gave interesting results. Severe symptoms including severe dwarfing of foliage and light fruit set

were always produced on the prune types of plums—Italian, Smith (? Italian), German. The Lombard plum had moderately severe foliage symptoms but apparently without injury to fruit set. On the two remaining *P. domestica* varieties—Bradshaw and Reine Claude—significant symptoms were not produced, although there appeared to be a slight reduction in size of foliage. Unfortunately, the planting containing these two varieties was lost before determining whether they were carrying the virus.

The results of the transmission tests between the prune and the *P. salicina* varieties of plums were apparently negative except for a slight dwarfing of foliage but difficult to diagnose because of the presence of other viruses.

In each of 3 separate nursery shipments of dormant trees of the Red June variety of plum one or more trees were found carrying a line-pattern virosis (2) when grown in the greenhouse. It was noted that the trees showing the chlorotic line patterns bore a lighter green foliage. Ordinarily, they showed the striking line pattern symptoms at the lower temperatures of spring, becoming masked with increase in temperature as the season advanced. The affected trees were otherwise apparently normal.

Occasional trees of the Abundance and Burbank varieties also showed a lighter green color of foliage and, since this abnormality also occurred in the checks, it could not be attributed to the prune-dwarf virus, also being presumably a contamination originating in the nursery. Incidentally, one Abundance tree had what was considered a typical line pattern, much fainter in outline than that seen on Red June. Unfortunately, for lack of space, these experiments were terminated without determining whether or not the *Prunus salicina* varieties were carrying the prune-dwarf virus.

As already mentioned, Damson plums (*P. insititia*) from several sources were found to be masked carriers of the prune-dwarf virus. The lesson to be drawn from the above transmission experiments on plum clearly indicates the importance of growing, observing, and indexing all test plants on other *Prunus* spp. before using them in host-range studies. The recent report from Canada (1) cites two good cases at point. It is further indicated that failure to show symptoms is not a reliable criterion for absence of virus.

Plum to Cherry

The results of transmission tests from plum to cherry have apparently all been negative. Two sour-cherry varieties (Early Richmond and Montmorency) and 4 sweet cherries (Black Tartarian, Napoleon, Yellow Spanish, and Windsor) were employed. For each variety 3 plants received 2 buds each from diseased prune, 1 plant 2 buds from healthy prune, and the fifth plant was not budded. At the end of 2 years no symptoms were visible on these plants, with one exception. A single shoot on one of the Black Tartarian trees showed a marked dwarfing of leaves and stunting of growth. The growth was so short that a bud could not be taken for transmission purposes.

The identity of a chlorotic ring-spot and line-pattern disease, which ap-

peared on a Mahaleb root sprout from one of the Montmorency trees, was not determined because it failed to produce symptoms when budded into peach seedlings. This latter disease was observed again in 1941 in a cherry nursery on Mahaleb suckers coming up from roots where sour-cherry buds failed to take the previous season. Whether or not it was responsible for these failures is not known.

Plum to Peach

Transmission to healthy Elberta peach trees was effected by grafting from diseased prune in 5 separate experiments, all but one of which were

FIG. 3. Early-stage symptoms of prune dwarf on newly developed terminal foliage of Elberta peach, consisting of leaf distortion, wavy margins, and a chlorosis of the distal half, where deformation was most marked. This tree was budded in September, then placed outdoors till mid-January. The photograph was taken in February, about 1 month after the trees had been returned to the greenhouse (65° F.) for forcing.

conducted in the greenhouse. The outdoor experiment was conducted in the writer's garden under good growing conditions. Therefore, failure to transmit this disease in earlier attempts (7) was very probably due to poor growing conditions rather than immunity on the part of the plant. Since the results from these experiments were very similar, only two will be discussed here, the third and fourth, conducted, respectively, in the greenhouse and outdoors.

The third experiment, begun on August 31, 1936, employed 39 2-year-old

Elberta peach trees growing in pots, 10 receiving 2 buds each and 19 one bud each, the remaining 10 being held as checks. To verify the presence of prune-dwarf virus in the budwood 4 Italian prune trees also were included, each receiving 2 buds. After budding, the trees were placed outdoors. They became dormant about November 1, without showing any symptoms.

The first symptoms suggesting transmission of the dwarf disease to peach were noted on February 17, 1937, or about a month after the trees had been moved from outdoors to the 65° F. greenhouse on January 15, and con-

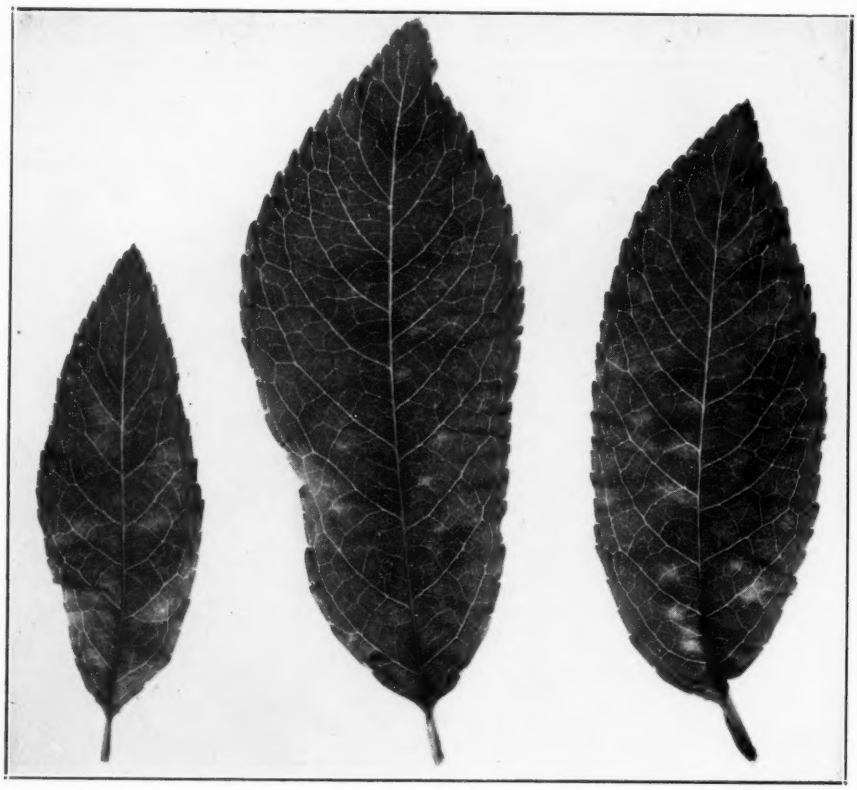

FIG. 4. Symptoms of prune dwarf on Elberta peach, which followed about 10 days after the stage shown in figure 3. The chief characteristics were chlorotic spots, mild crinkling and shortening of the leaf blade, the effect being most pronounced in the basal half of the older leaves.

sisted mainly of leaf distortion (Fig. 3). Ten days later occasional chlorotic spots (Fig. 4) appeared on several but not all of the leaves. The distortion shown in the figure was largely confined to the distal halves of the younger leaves, which became narrower, wavy margined and lighter green, whereas the chlorotic spots appeared more commonly on the older more nearly normal shaped leaves. Ten out of the 19 trees receiving one diseased bud and 9 out of the 10 trees receiving two buds showed these symptoms on February 27. As the season advanced the greenhouse temperature was raised to 70°

F. and above. With the rise in temperature the affected leaves gradually recovered from the two types of chlorosis and from much of the distortion, except the waviness of the leaf margin. The next development, especially

FIG. 5. Prune dwarf on peach showing typical rosetting and wavy leaf on a 4-year-old Rochester peach tree grown in fertile garden soil. The tree was planted as a 2-year-old in 1935, received four diseased buds from prune in 1936, and was photographed in 1937. The dead twigs were the result presumably of winter injury.

on the plants receiving 2 diseased buds, was a stunting of shoot growth accompanied by a variable amount of leaf drop and marginal leaf spot on the older leaves, and a tendency for the shoots to develop rosette similar to

but milder than that induced by rosette-mosaic on peach (3). Later, with the coming of summer temperatures, the majority of the affected plants had largely recovered from the more conspicuous of the symptoms. A year later these trees appeared to have almost completely recovered from the disease, except possibly for a slight waviness of leaves and stunting. On March 20 the 4 check prune trees were showing typical early-stage symptoms ranging from chlorotic ring spots of the older leaves to willowing of the tip leaves on the terminals.

An interesting angle of this experiment was the fact that nearly all of the peach trees, even though only beginning their third season of growth, blossomed, and a moderate number bore fruit to maturity, thus affording an opportunity to study fruit symptoms. Five of the diseased trees matured fruit in June. In every case the suture side developed slower than other parts of the fruit, was slightly rough and off shape, and, instead of yellow with a red blush, as in the checks, was greenish-yellow, which contrasted with the prevailing naphthalene-yellow over the remainder of the fruit. The fruits on affected plants were smaller and matured from 7 to 15 days later than those on the check plants.

The outdoor experiment was conducted on 2 trees each of 3 peach varieties—Elberta, Rochester, and South Haven. These trees were planted when 2 years old on May 2, 1935, and were making excellent growth when 1 tree of each variety was grafted with 4 diseased buds on August 6, 1936. All 3 budded trees were showing symptoms in 1937, the most distinctive being a stunting of the terminal growth, simulating rosette and the tendency of the leaves to have wavy margins (Fig. 6). It will be remembered that these same symptoms were the end result of the greenhouse experiment just described. The following year these trees likwise seemed to have recovered from the disease.

RESEMBLANCE TO ROSETTE-MOSAIC DISEASE

Because these symptoms, although milder, bore a resemblance to the rosette-mosaic disease on peach, comparative studies were undertaken with a view to clarifying their relationship (3). Although this study has not been concluded, the results to date suggest that if a relationship exists the virus causing rosette mosaic is a much more aggressive strain on the peach than is prune dwarf, whereas the latter virus is most aggressive on the prune. The recent unreported finding by Cation in Michigan of a mild strain of rosette-mosaic virus may have a bearing on this point since it suggests the existence of strains within that virus.

SUMMARY AND CONCLUSIONS

Originally found only in Niagara County, New York, prune dwarf is now known to be present also in Canada.

The Damson plum is known to harbor the prune-dwarf virus without showing symptoms, and the causal virus may be more widespread than is realized because of this fact. Three out of five nursery and orchard sources of Damson plums were positive for the virus when indexed on Italian prune.

Two severe infestations of Damson plum with this virus have been reported in Canada, where the growers top-worked to the susceptible Italian prune. Symptoms of prune dwarf are also masked by Bradshaw plum.

The disease spreads slowly and, ordinarily, to the immediately adjacent trees in orchards but in spurts which have been correlated with severe infestations with the green plum aphid. Insect transmission experiments, however, have all been negative thus far.

Fruit yields on the prune types of plums are extremely reduced, averaging less than 10 per cent of normal. The Lombard plum, which suffers foliage symptoms but not the abortion of pistils, yielded slightly below normal, apparently because of the reduced foliage. Fruiting of the Damson plum, which masked symptoms, seemed to be normal.

The only essential for successful transmission to susceptible plants was tissue union between cion and stock. The shortest incubation period for prune was 5 weeks. Of the *Prunus domestica* varieties tested, only Lombard plum, besides the prune types, developed typical foliage symptoms. None of the *P. salicina* varieties developed marked symptoms, but it was found that a distinct chlorotic line pattern virus was prevalent in Red June and also present in Abundance. The Damson plum (*P. insititia*), while failing to develop typical foliage symptoms, was, subsequent to budding, found to contain the virus. Transmission tests from prune to cherry have all been negative. The peach was found susceptible to prune-dwarf virus with the production of a number of foliage symptoms from which the plants largely recovered as the season advanced. The peach fruit on affected plants was retarded in development, especially on the suture side. The symptoms that appeared in both outdoor and greenhouse tests with Elberta peaches were a stunting in terminal growth, simulating a mild case of rosette and the tendency toward wavy margins for some of the leaves. By the following year the affected peaches had largely recovered from the disease.

Studies are under way to determine what relation, if any, exists between prune dwarf and peach-rosette mosaic, to which it bears a definite resemblance in symptoms on peach.

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A ROOT ROT OF COTTON CAUSED BY THIELAVIOPSIS BASICOLA

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(Accepted for publication February 3, 1942)

INTRODUCTION

In 1922 a peculiar root rot of cotton plants was observed at the U. S. Field Station, Sacaton, Arizona. Affected plants were characterized by main roots, swollen at the collar and manifesting an internal purplish-black discoloration. It was first thought to be a disease that appears on mature plants in late summer or early fall, and was described as an internal collar rot (3). Later, it was observed in the spring destroying seedlings 3 to 4 weeks old. During the hottest part of the season it is not evident. It reappears with the approach of fall, when further mortality of cotton plants occurs (Fig. 1, A). It was noted early that the disease affected American-Egyptian cotton more severely than it did the upland type. It also showed great persistence in poorly drained soils, and recurred in cotton after several years of rotation with alfalfa and other nonsusceptible crops. In 1940 it was found in a commercial field of American-Egyptian cotton in the Upper Gila River Valley, nearly 200 miles east of Sacaton, but in the same drainage area and soil type. In 1938 the causal organism was identified as the fungus *Thielaviopsis basicola* (Berk. and Br.) Ferraris, cause of black root rot of tobacco in Europe and in some of the eastern and mid-western States of the United States.

For many years the fungus deemed responsible for black root rot of tobacco carried the name *Thielavia basicola* Zopf. The work of McCormick (5) in 1925 furnished evidence that *Thielaviopsis basicola*, which usually develops two spore forms, endoconidia and club-shape chlamydospores, is the actual cause of black rot. An ascogenous fungus, frequently associated with it, and long believed to be a form of the causal organism, was given the designation *Thielavia basicola*. The latter is not known to be parasitic. In cultures it has a tendency to produce perithecia, especially if grown in the presence of *Thielaviopsis* or certain other fungi. *Thielavia* has not been observed in association with *Thielaviopsis* on cotton in Arizona.

The report in 1939 of an internal collar rot of cotton in Arizona, caused by *Thielaviopsis basicola* by King and Barker (4), apparently is the first record of this fungus in the arid Southwest, and also of its damage to cotton under field conditions. Smith (9), Rosenbaum (7), and Johnson (2) previously had reported it as causing a root rot of cotton under controlled experimentation; and, in 1940, Sherbakoff (8) reported it as responsible for injuries to cotton seedlings at Knoxville, Tennessee. The disease is not

[1] The writers gratefully acknowledge the assistance of Lloyd A. Brinkerhoff in laboratory and greenhouse work connected with this investigation while employed as Agent at the U. S. Field Station, Sacaton, Arizona, December 1, 1939, to April 12, 1940, and to H. D. Barker for many helpful suggestions.

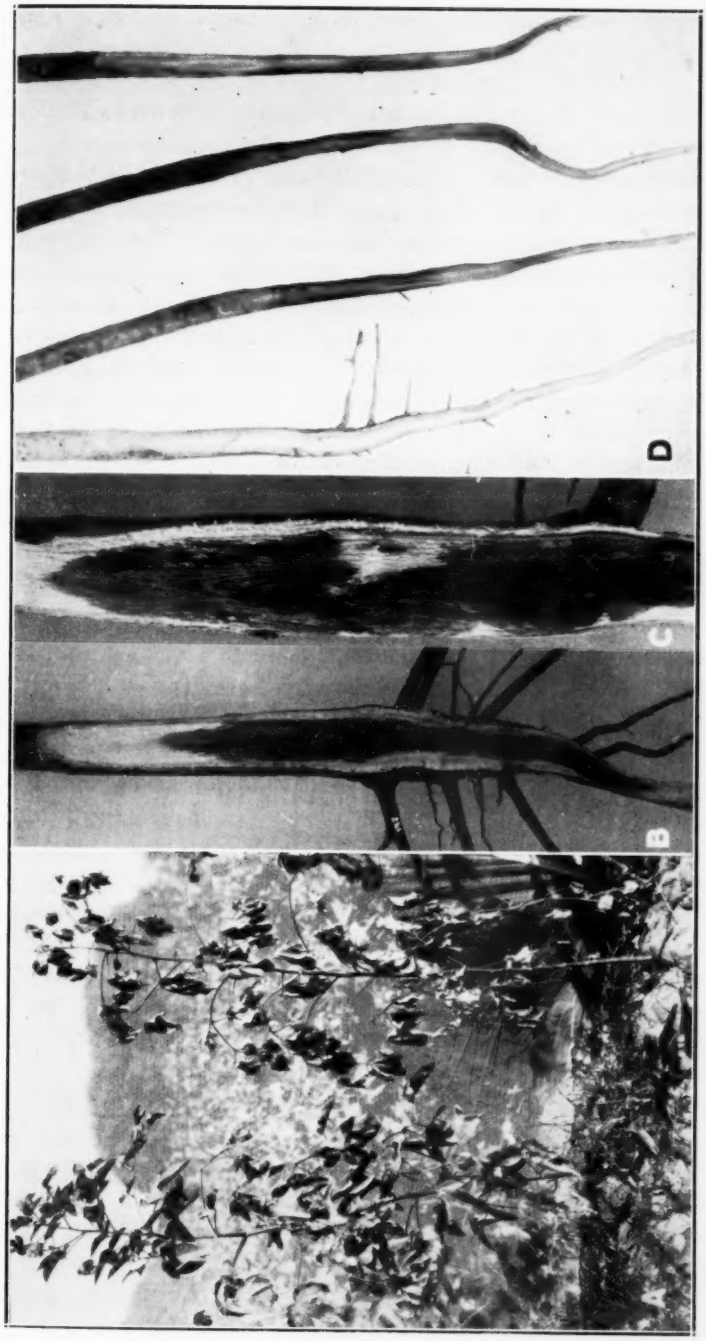

FIG. 1. Symptoms of Thielaviopsis root rot on Pima (American-Egyptian) cotton plants. A. Plant at right dying late in the season; plant at left not affected. B. and C. Internal collar rot symptoms. Note swelling at the crowns. D. Three taproots at right show typical injuries to seedlings about 6 weeks old. Root at left not affected.

believed to be of common occurrence in Arizona, but its discovery in two widely remote locations on the Gila River suggests that it may be indigenous.

HISTORY

After discovery of internal collar rot of cotton at Sacaton in 1922, specimens of affected roots were sent to several pathological laboratories, but no parasitic organisms were isolated. In 1924 G. L. Peltier, while temporarily employed in Arizona by the Bureau of Plant Industry, examined some affected plants and prepared a preliminary description of the disease, which remained unpublished pending results of some culture work he was unable to complete before resuming his regular duties in Nebraska. In later years several unsuccessful attempts were made at Sacaton to isolate the causal organism. In January, 1938, it was observed that the discolored root tissues, particularly the conducting vessels, invariably showed numerous dark colored chlamydospores. This discovery aided in the selection of recently invaded tissues and the isolation of the organism, which formed the dark-brown chlamydospores. Inoculation tests proved that this was the causal organism.

Cultures of the fungus were submitted to the Division of Mycology and Disease Survey, Bureau of Plant Industry, and were identified tentatively by J. A. Stevenson as *Thielaviopsis basicola*. For comparison, type cultures of *Thielaviopsis basicola* were obtained from the tobacco experiment station at Greenville, Tennessee, and from Washington University, St. Louis, Missouri. Comparative cultural and inoculation studies indicated that the Arizona organism and the tobacco isolates belonged to the same species, but that they differed slightly in cultural characteristics.

SYMPTOMS AND EFFECTS

When the disease is studied on cotton plants in the seedling stage, the aboveground symptoms somewhat resemble the effects of root-knot nematodes or injuries from toxic salts: The seedlings are stunted and apparently unhealthy; the leaves are often small and cupped, pale green, purplish along the veins, and show marginal browning and burning. Gradually the lower leaves wither and drop off; many seedlings succumb at various stages between the development of the 3rd and 6th leaves.

The roots of the affected seedlings show a purplish-black discoloration of the affected tissues (Fig. 1, D). As in the case of mature plants, the central cylinder usually shows the first effects of invasion by the fungus. When the main root of a seedling with mild symptoms is split, it is observed that the stele already is discolored and often portions have disintegrated to form cavities. In plants with more severe symptoms the roots show portions of the cortex in a black, shrunken condition. By the time the plants are dead nearly all of the cortex, as well as the stele, is involved, and the entire root is so fragile that little remains with the plant when it is pulled from the ground. As temperatures rise with the advance of summer, the affected

young plants that have survived begin to recover; by mid-summer there is little evidence of the disease. On cutting through one of the recovered plants at the collar, a cylinder of necrotic tissue surrounded by healthy tissues is usually found. When lower temperatures prevail in late summer the infection extends from these occluded lesions and effects further damage to the plant, often causing its death.

The symptoms shown by mature plants when they wilt and finally die might easily be mistaken for Phymatotrichum root rot (Fig. 1, A). It is to be noted, however, that the leaves remain on the dead plants longer than in the case of death from most of the cotton diseases. Also, it is characteristic of occasional mature plants to die. It appears probable that many of these may be plants in which there is renewed activity of the organism in old lesions, although some of them may represent new infection that has developed after temperatures again become favorable. It is seldom that more than 2 or 3 plants die in one group.

In mature plants the first sign of the disease is an abnormal swelling of the main root, beginning at the collar and extending 3 to 6 inches below the ground line (Fig. 1, B). Usually, the bark tissue on the tap root appears healthy, except that the lenticels often are distended and occur as cork-like protuberances. Splitting the tap root reveals a highly colored, brown to purplish-black, heart rot affecting the entire stele of the swollen area (Fig. 1, C). The discolored tissues for the most part are firm, with only a few small cavities to indicate a slow, dry-rotting process. The line of demarkation between the white healthy tissues and the discolored, diseased tissues is very distinct. The discoloration seldom extends far above the ground level. Immediately after the death of the plant, the only evidence of external decomposition is noted on the ends of the lateral roots and far down on the tap root. Here the entire root is involved and the bark may slough off on handling. Later, the cortex of the swollen area on the main root is broken through and black sunken areas appear on the outside. A general decay then occurs from attack of secondary organisms.

On diseased seedlings and occasionally on mature plants, the lateral roots may be finer and more numerous than on normal plants. This behavior is not so pronounced as that described for the tobacco plant.

THE ORGANISM

It was found that a microscopic examination of thin sections of discolored tissues provides an easy and reliable identification of the disease. Tissues, discolored by *Thielaviopsis*, invariably contain many brownish, multisegmented chlamydospores (Fig. 2, A). Chlamydospores abound in the conducting vessels and in cavities and intracellular spaces of the phloem, xylem, and medullary-ray structures. Occasionally, they may be seen within parenchyma cells. Endogenous spores rarely are seen within the host tissues. In their restricted positions within the tissues the chlamydospores do not attain the typical club shape, the regularity in number of

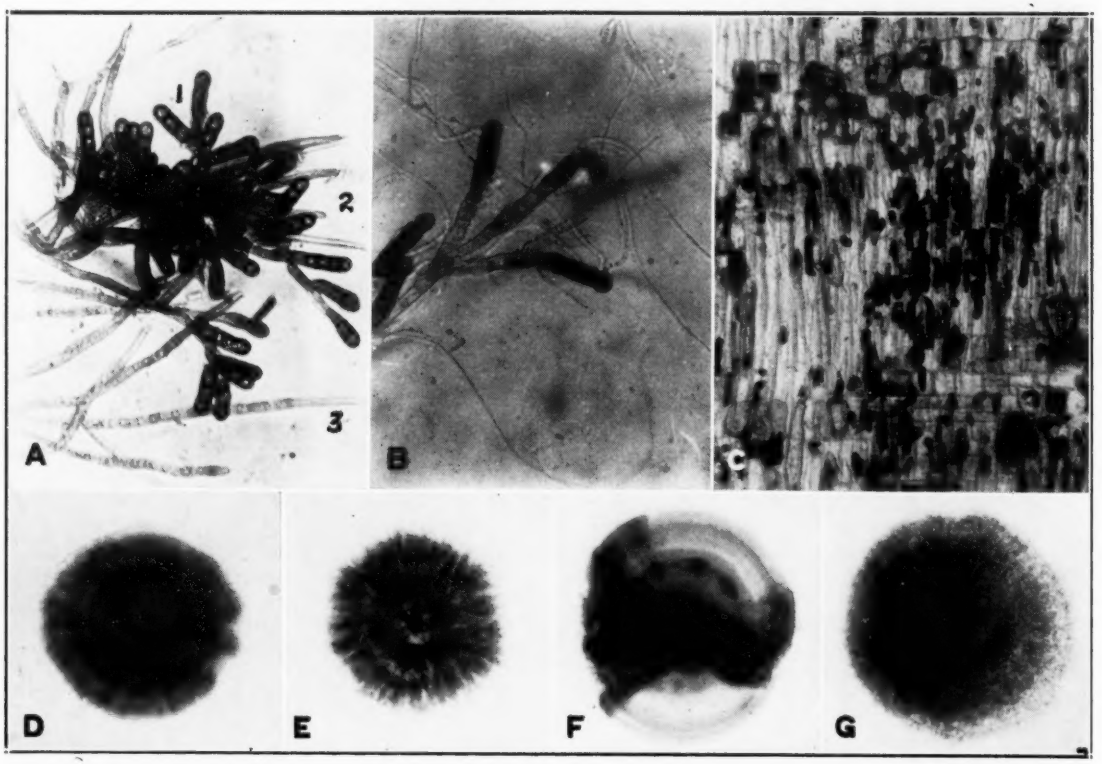

FIG. 2. Some characteristics of *Thielaviopsis basicola*. A. Types of spores produced in artificial culture: 1, Chlamydospores; 2, endospores; 3, endoconidiophore. B. Chlamydospores germinating. ×approximately 600. C. Chlamydospores within the vascular tissues—(longitudinal section of Pima root crown). D and F. Colonies of the cotton isolate (Arizona): D, 9-day-old culture on potato-dextrose agar; F, 12-day-old culture on onion agar. E and G. Colonies of the tobacco isolate (Missouri): E, 9-day-old culture on potato-dextrose agar; G, 12-day-old culture on onion agar.

segments, and the racemose arrangement on the hyphae, as is common on artificial media (Fig. 2, C). Usually they are cylindrical and consist of from 3 to 14 segments extending in chain-like formation through the vessels.

Cultural Characteristics

The Arizona Thielaviopsis fungus is readily isolated from cotton plants affected with black root rot, and can be grown on a wide range of culture media. On agars containing malt extract, potato dextrose, or decoctions of onion or of *Nicotiana glauca* the growth is vigorous, with endoconidia forming abundantly within 2 days and chlamydospores within 4 days after incubation at room temperature. On potato-dextrose agar the initial growth of the colonies is ashy-gray. Except at the margins, it soon becomes a blackish-brown imparted by the chlamydospores.

Comparisons were made of the growths of the Arizona cotton isolate and the tobacco isolate from Missouri grown on different media and at different temperatures. When grown on the same plates or in parallel series of plates on potato dextrose agars and incubated at 16, 21, 26, and 30 degrees C. the growth rates were about equal for both isolates, with most rapid growth occurring at 30° C. The average daily growth for both isolates at this temperature for the first 8 days after inoculation was 3 mm. Gilbert (1) reports 30° C. as the optimum temperature for growth of *Thielaviopsis basicola*. Rawlings (6), however, considered the optimum for 3 races of the fungus in his study as near as 20° C.

While the two isolates reacted much alike at different temperatures, they were easily distinguishable in appearance (Fig. 2, D and E). On potato-dextrose agar the colonies of the Arizona cotton isolate were lighter in color, denser, and more submerged than those of the tobacco isolate. Concentric zonation was pronounced in both, but more conspicuous in that from tobacco. No sectoring occurred on any of the cultures on potato dextrose. Johnson and Valleau (3), however, observed instability and sectoring in all of 11 tobacco isolates grown on potato-dextrose agar. On onion agar both the Arizona and Missouri cultures grew vigorously, but the mycelium of the cotton isolate when several days old was darker and more appressed (Fig. 2, F and G). Sectoring was a common tendency with the cotton isolate on this medium, but did not occur in that from tobacco. Transfers were made from one of the white or albino sectors and one of cinnamon-buff color. Both maintained these characteristics during several transfers on sweet-potato plugs and potato-dextrose agar. It seems significant that Rawlings (6) observed a tendency for his Texas isolate from cotton to develop sectors, particularly on onion agar, while an isolate from tobacco in Tennessee and one from *Primula obconica* in Holland failed to develop sectors on potato-dextrose, onion, or Leonian's agar.

Measurements of the endospores and chlamydospores of the two isolates showed no significant differences in size. The mean length of the endospores was approximately 14.5 μ and mean width 4.4 μ. The chlamydospores averaged 38 μ in length and 11 μ in width.

One noticeable difference was in the coloring of the hyphae bearing the chlamydospores. In the tobacco isolate the spore-bearing branches and basal cells of the chlamydospores usually were hyaline; whereas the brownish coloration of the isolate from cotton often extended into these structures.

Some germination tests were made with the endospores and chlamydospores of the cotton isolate of the Thielaviopsis fungus. In hanging drops of sterile tap water or on cubes of potato-dextrose agar a large proportion of the endospores germinated within 16 to 20 hours. The chlamydospores, however, failed to germinate under these conditions. When planted on dilute drops of malt agar containing a trace of pepsin, about 40 per cent of the chlamydospores developed germ tubes from one or more segments (Fig. 2, B).

INFECTION EXPERIMENTS

In 1939, inoculations with pure cultures of *Thielaviopsis basicola* were made on a small number of cotton plants in the field and greenhouse at 4 different times of the year. The varieties represented were Pima, Sea Island, and Sakellaridis of the Barbadense species and Acala of the Hirsutum species. In all cases the plants had reached the fruiting stage when inoculated. Longitudinal incisions with a sterile scalpel were made on the tap root just below the ground level. Tufts of spore-bearing mycelium were inserted and the incision was closed with adhesive tape. Half as many control plants received similar treatment, except for insertion of inoculum. The first examinations were made about 20 days after inoculation. With few exceptions the inoculated plants showed discoloration in the vascular tissues extending about 7 cm. from the point of inoculation on varieties of the Barbadense species, and about 4 cm. on varieties of Hirsutum. After 20 to 30 days chlamydospores were always observed within the discolored tissues, and a swelling of the tap root near the crown was often noticeable on plants of the Barbadense species but not on those of the Hirsutum species. A few of the plants were dwarfed and apparently unhealthy, but none of them died. The incision on the control plants healed rapidly, and discoloration failed to develop in the vascular cylinder.

In December, 1939, more elaborate greenhouse inoculation experiments were undertaken for comparing the pathogenicity of the Arizona cotton isolate and the tobacco strain from Missouri. Pima cotton and Maryland broadleaf tobacco plants were chosen for hosts. The latter were grown in crocks filled with clay loam. Some of the cotton was grown in soil, and some in sand nutrient cultures. The inoculum, consisting of pure cultures grown on sterilized cotton roots, was inserted in holes made in the soil or sand between the plants when the seedlings were only 2 weeks old. Within 9 days after inoculations were made on December 27, the tobacco seedlings began dying in all of the containers inoculated with either the cotton or the tobacco isolate, whereas the controls remained healthy. During the following 2 weeks more than one-third of the inoculated tobacco seedlings died from black root rot. Invariably, chlamydospores could be found in the

diseased tissues. No further mortality of tobacco plants occurred; but, when final examination was made on March 25, all plants in inoculated containers showed damage from black root rot (Fig. 3, D–F).

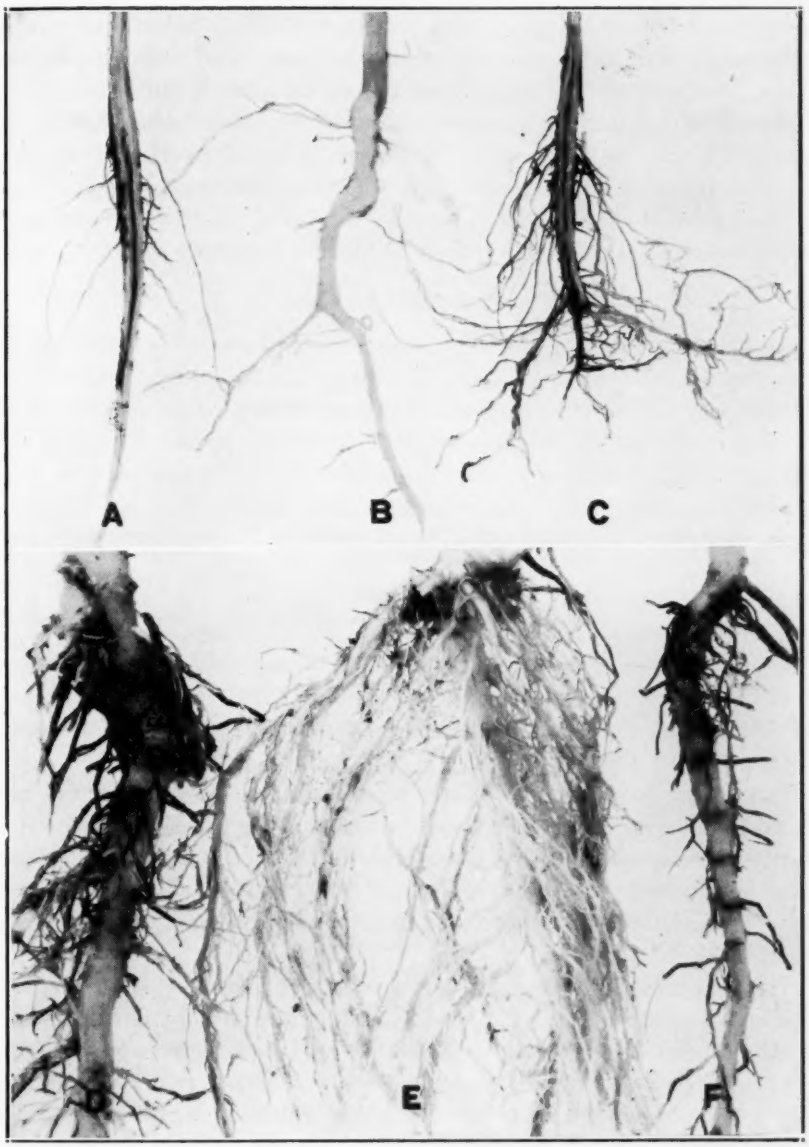

FIG. 3. Cross inoculations with the cotton and tobacco isolates. Pima cotton inoculated with the cotton isolate (A) and tobacco isolate (C). Maryland Broadleaf tobacco inoculated with the cotton isolate (D) and the tobacco isolate (F). Control plants: B, cotton; E, tobacco.

Thirty-seven days after inoculation, a Pima cotton plant was found dead in a sand-culture container inoculated with the cotton isolate, and another

in a container inoculated with the tobacco strain. At that time nearly all plants in the inoculated sand cultures showed disease symptoms, and many of the roots were blackened and rotted; only 4 plants finally died. In the soil cultures the Pima cotton plants showed no definite disease symptoms aboveground before March 5. On that date 2 plants inoculated with the cotton isolate were wilted and were found to have rotted roots, but they subsequently recovered. Nearly all of the cotton plants grown in soil and inoculated with either isolate were unhealthy and had diseased roots when examined March 25, but none of them died (Fig. 3, A–C). It appeared that the conditions in the greenhouse were not continuously favorable for optimum development of the fungus in the cotton plants, and the disease symptoms were not so severe on them as it often is under field conditions.

Cross Inoculations

On February 21, both the tobacco and the cotton isolates were reisolated from diseased tobacco seedlings in the greenhouse and cultured on sterilized cotton roots. On February 29, both reisolates were used to inoculate 60-day-old Pima cotton plants grown in soil in the greenhouse in large flats. None of the plants died, but examinations made on March 22 showed similar and about equal damage to the roots from the two fungus isolates. Chlamydospores were abundant in the discolored root tissues inoculated with either.

SUMMARY

A new disease of cotton characterized by a swollen tap root near the collar and an internal purplish-black rot of the vascular tissue was observed at the U. S. Field Station, Sacaton, Arizona, in 1922. The causal organism was isolated in 1938 and identified as the fungus *Thielaviopsis basicola* (Berk. and Br.) Ferraris.

The disease occurs in two widely separated areas in Arizona, but is not believed to be extensive. It persists in the soil year after year, even in the absence of cotton culture. Its spread through the soil is slow and its damage to the cotton crop is not severe, except in occasional seasons. American-Egyptian varieties of cotton proved more susceptible to it than did upland varieties.

The disease is most damaging to the cotton crop in the seedling stage. It sometimes destroys stands of American-Egyptian cotton, and retards the growth of surviving seedlings. Affected seedlings show a characteristic purplish-black dry rot in the central cylinder of the root, where cavities often form. Many seedlings recover when high temperatures prevail, the internal lesions becoming surrounded with healthy tissues. In the fall the occluded lesions may become active and often kill the mature plants.

The disease is readily identified, since microscopic examination of thin sections of the discolored tissues almost invariably reveals the presence of brownish chlamydospores in the conductive tissues.

In cultures on various media the fungus isolated from cotton resembled

in appearance and behavior isolates from tobacco obtained from Tennessee and Missouri. Slight differences in color and density of the colonies were apparent on all media, and, on onion agar, the development of white or buff sectors occurred frequently in the cotton isolate but not in the tobacco strain.

Inoculations and cross inoculations on American-Egyptian cotton and Maryland Broadleaf tobacco plants, under greenhouse conditions, were successful with both the cotton and tobacco isolates. Nearly all of the inoculated plants showed symptoms of the disease, either externally or internally, but only a few of them died.

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GALL FORMATION BY PHYTOMONAS TUMEFACIENS EXTRACT AND INDOLE-3-ACETIC ACID IN CULTURES OF TOMATO ROOTS[1]

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INTRODUCTION

Since 1936 reports have appeared in which galls incited by *Phytomonas tumefaciens* (Sm. and Town.) Bergey *et al.* have been compared with those stimulated by extracts of this pathogen and by indole-3-acetic acid. The morphological (3, 5, 7, 9), histological (5, 9, 12), and biochemical (11) similarities of these tumors have been observed. Growth hormone is produced in cultures of *P. tumefaciens* and many bacteria and fungi (2, 3, 12, 13, 19, 21), and extracts of the cultured media of the crown-gall organism have given chemical tests for indole-3-acetic acid (12). With a reported exception (15), crown-gall tissue has yielded higher concentrations of phytohormones (auxin a, auxin b, and indole-3-acetic acid) than normal tissue (11, 12, 13). All 3 auxins have been isolated from healthy higher plants (2, 8, 21). In addition, there has been observed a significant increase in the auxin content of legume nodule tissue (10, 20), and of smut-gall tissue of corn.[3] Such results have led to the opinion that the growth of galls, and similar pathological processes, may be related to disturbed hormone balance in the plant (7, 10, 11, 12, 20, 21).

In the present investigation root tips grown *in vitro* were employed as these plant cultures have not previously been used to study gall formation by indole-3-acetic acid and *Phytomonas tumefaciens* extracts. Furthermore, indole-3-acetic acid was employed to ascertain the relationship of growth to tumor development, and to throw light upon a disputed point (2, 4, 6, 14, 16, 21, 23), namely, whether in low concentrations this chemical stimulated or inhibited the growth of roots.

EXPERIMENTAL METHODS AND RESULTS

General Procedures

A strain of excised tomato roots (*Lycopersicum esculentum*) was used upon which considerable physiological data were available (22, 23), and which was very generously supplied by P. R. White. The yeast extract-salt-sugar solution devised by this investigator was employed. In experiments III and IV (Table 1) a synthetic nutrient medium was used in which

[1] An abridgment of a dissertation in the Department of Bacteriology submitted by the senior writer to the faculty of the Graduate School of Arts and Science of New York University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Degree conferred June, 1941.

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[3] Moulton, J. E. Doctor's Thesis. University of Chicago, 1941. Quoted by Link and Eggers (11).

TABLE 1.—*The effect of indole-3-acetic acid upon the growth, number of secondary roots formed, and the dry weight of excised tomato roots*

Number of root cultures per concentration	Concentration (mg./l.) of indole-3-acetic acid											
	0		0.00125		0.0125		0.025		0.05		0.5	
	L.[a]	B.[b]	L.	B.	L.	B.	L.	B.	L.	B.	L.	B.
Experiment I												
15	52[c]	4	46	7	19	4	15	6	2	3	1	5
Experiment II												
20	58	7	35	6	17	5	3	1	4	2	0	1

Number of root cultures per concentration	Concentration (molar) of indole-3-acetic acid									
	0		10^{-14}		10^{-12}		10^{-10}		10^{-8}	
	L.	B.	L.	B.	L.	B.	L.	B.	L.	B.
Experiment III[d]										
50	83	14	.....	.....	71	12	71	11	26	5
Experiment IV[d]										
80	71	10	72	12	75	10	73	11	29	4
Total dry wt. (mg.) Exp. IV	100.3		101.0		100.4		89.6		78.2	

[a] In all tables L. indicates the final length of roots, including length of branches, minus initial length, expressed in millimeters.

[b] In all tables B. indicates the final number of branches formed minus the initial number.

[c] The figure 52 represents the average increase in length in millimeters of 15 roots grown in nutrient solution to which no indole-3-acetic acid had been added (control roots). Similarly, in all tables each number represents an average for each concentration employed.

[d] In synthetic medium.

0.5 mg. thiamin and 1.0 mg. glycine per liter were substituted for the yeast extract in the aforementioned medium.

In subculturing, root tips about 15 mm. long were cut with scissors and removed by transfer needles to 125-ml. Erlenmeyer flasks containing 50 ml. fresh sterile nutrient solution. The root cultures, one to a flask, were kept in dim light at room temperature, and subcultured weekly. Growth was measured by the increase in length measured by millimeter ruler, by the increment in dry weight, and by the macroscopically observed increase in number of secondary roots. In order to obtain greater accuracy in experiments III and IV (Table 1), the rootlets were enumerated under magnification ($\times 100$). To determine the dry weight, roots were oven-dried and weighed on an analytic balance. The hydrogen-ion concentration (pH) of culture solutions was measured electrometrically.

To make histological preparations, roots were killed and fixed by Navashin's solution, dehydrated by ethyl alcohol-xylol mixtures, embedded in

paraffin, sectioned at 10 μ thickness, and stained by Heidenhain's iron-haematoxylin technic.

Cultures of *Phytomonas tumefaciens* were received from A. J. Riker and E. A. Siegler, to whom we acknowledge our thanks. Galls were formed by these cultures following needle inoculation into stems of young tomato plants, grown at the Brooklyn Botanic Garden through the courtesy of G. M. Reed.

Effect of Culture Extract of P. tumefaciens

The microorganisms were grown 3 days in 6.5 liters of potato-dextrose broth. The culture medium was then passed through a Berkefeld N filter to remove the bacteria. The filtrate was evaporated under reduced pressure to a volume of 100 ml., acidified to pH 3, and extracted 7 times with 0.5 volume of peroxide-free ether. The ether layers were combined and allowed to evaporate at room temperature. The ether residue was added to nutrient fluid in 20 flasks in concentrations of 150 to 1.5×10^{-1} mg. per liter.

As a result, the ether extract reduced the growth of roots, several of the cut ends of which were swollen, and there was excess root hair development.

Effect of Indole-3-acetic Acid

Gross Appearance of Roots. Excised roots are white and translucent, slender and long, regular in shape, and float on the surface of the medium (Figs. 1, A, and 2).

Roots grown in nutrient solution containing indole-3-acetic acid in concentrations from 10.0 to 0.0125 mg. per liter are completely to slightly brown and opaque, stunted in length, thickened and irregular in shape, and submerged in the fluid. Branches appear even at the tip, and like the primary root are stunted in growth. The higher the concentration of the phytohormone, the greater the effect (Fig. 1).

Well-pronounced galls are formed on treated roots (Fig. 3). These are found generally in conjunction with root primordia or branches, but often without the presence of either. The overgrowths may appear on the primary root or branches, at the growing point, medially, or at the cut end.

Microscopic Appearance of Roots. Normal unsectioned roots are regular and of uniform appearance, with a moderate number of root hairs. The vascular stele is readily visible (Fig. 2). As seen from histological preparations (Fig. 4, A), the cells are rectangular, and regularly arranged. The epidermis consists of a single layer of cells. The cortex comprises 3 to 5 layers of parenchymous cells, and a single innermost layer, the endodermis. Underneath the endodermis, a single layer of pericyclic cells bounds the vascular stele. Secondary roots are initiated by meristematic activity of the pericycle.

Treated whole roots are highly irregular in shape (Fig. 3). There is excessive production of root hairs, the tips of which frequently are swollen, like balloons. In microscopic sections (Fig. 4, B–D) of roots treated with indole-3-acetic acid the cells of the epidermis are enlarged; in the higher

concentrations of the chemical there is much fragmentation. The cortex is enlarged because of cell hypertrophy, because of increased intercellular space resulting from necrosis and cell disarrangement and of some cellular

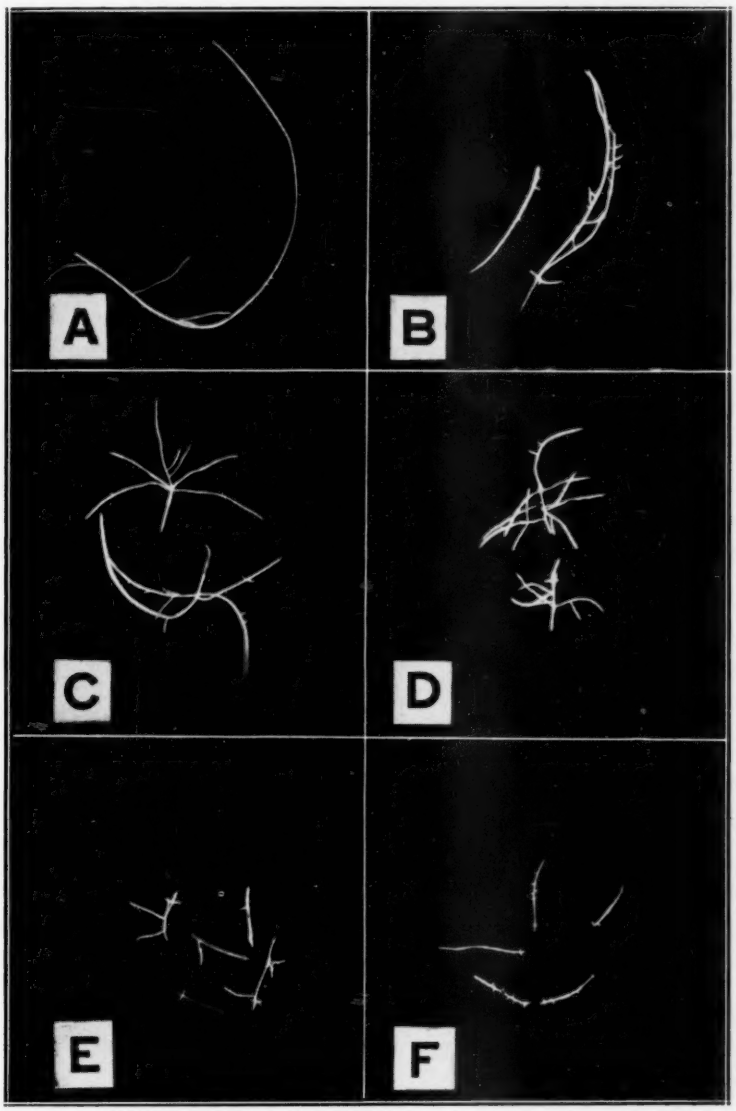

FIG. 1. Effect of the concentration of indole-3-acetic acid on growth of excised tomato roots. ×0.6. A. Normal root. B–F. Roots treated: B. 0.001 mg. indole-3-acetic acid per liter. C. 0.01 mg. per liter. D. 0.025 mg. per liter. E. 0.05 mg. per liter. F. 0.5 mg. per liter.

divisions (hyperplasia). There is intense stimulation of the meristematic activity of the pericycle (Fig. 4, B). Typical thickening in the absence of root initiation is shown in figure 4, D. In the cortex, particularly in the

pericycle, there occur multinucleate cells, most of which are binucleate. Xylem and phloem do not react strikingly to treatment. A comparison of figures 2, A, and 3, E, shows the effect of hormone upon the tips of primary roots. Compare figures 2, B, and 3, C, for the effect upon the cut ends of primary roots.

Growth of Roots. In the previous two sections the pathological effects observed were developed in dilutions of indole-3-acetic acid up to about 7×10^{-8} molar (0.0125 mg. per liter). Up through this dilution, and even to 7×10^{-9} molar, there was unmistakable inhibition of root elongation, reduction in the number of branches formed, and in the total dry weight (Table 1).

To determine if low concentrations of indole-3-acetic acid stimulated or inhibited growth, 2 additional experiments with 600 root cultures were performed. In the first, experiment III (Table 1), the roots grown in normal nutrient solution appeared to have increased in length (16 per cent), and to have formed more secondary roots. In the second test (Experiment IV,

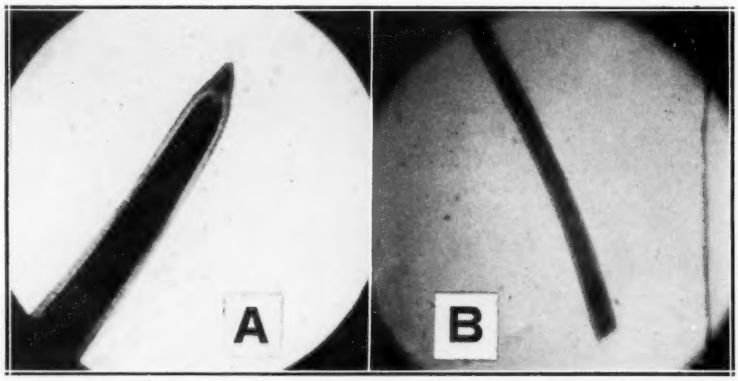

FIG. 2. Normal untreated roots. A. Tip of primary root. ×60. B. Cut end of primary root. ×24.

Table 1), a concentration of 10^{-12} molar indole-3-acetic acid seemed to accelerate elongation (6 per cent). Dry weight measurements, however, gave almost the same readings for the control, 10^{-14}, and 10^{-12} molar chemical-treated roots, respectively 100.3, 101.0, and 100.4 mg.

Toxicity of Indole-3-acetic Acid and Growth of Galled Roots in Normal Solution. An experiment was performed to determine the toxic range of indole-3-acetic acid. After a week's cultivation, the roots were transferred to plain nutrient medium (Table 2). It may be observed that a concentration of 5 mg. per liter is just about lethal, as there is neither elongation nor root initiation, and in addition, only 1 of 10 roots grew after transfers in normal solutions during a period of several weeks.

From the preceding experiment, 6 roots bearing prominent swellings were selected and cultured for 8 weeks in solution containing no indole-3-acetic acid in order to determine whether tumor tissue would grow or would affect the development of new tissue. However, the gall tissue persisted without apparent enlargement and all new growth of galled roots was normal.

Effect of Tryptophane, Indole, Acetic Acid, and pH. As indole-3-acetic acid is an intermediate product in the transformation of tryptophane to

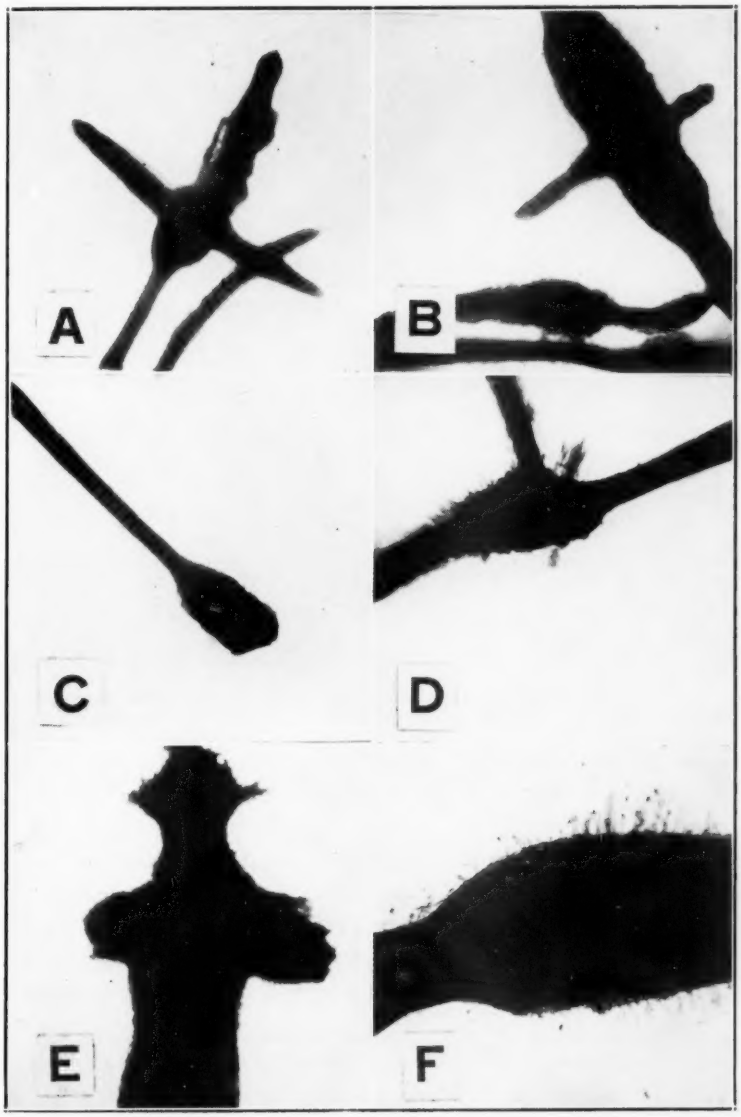

FIG. 3. Galls produced on excised tomato roots by indole-3-acetic acid. A. Root tip and branches in 0.05 mg. indole-3-acetic acid per liter. ×24. B. Root tip and branches in 0.025 mg. per liter. ×24. C. Cut end of primary root in 0.05 mg. per liter. ×24. D. Medial swelling, showing branch and excess root hairs. Root in 0.05 mg. per liter. ×24. E. Root tip treated with 0.5 mg. per liter (retouched). ×60. F. Middle section of root in 0.5 mg. per liter (retouched). ×60.

indole by microorganisms (2, 21), these substances were tested for their possible carcinogenic effect upon 78 roots. With tryptophane (10^{-4} to 10^{-9} molar) a few swellings resulted, but neither hypertrophy nor initiation of

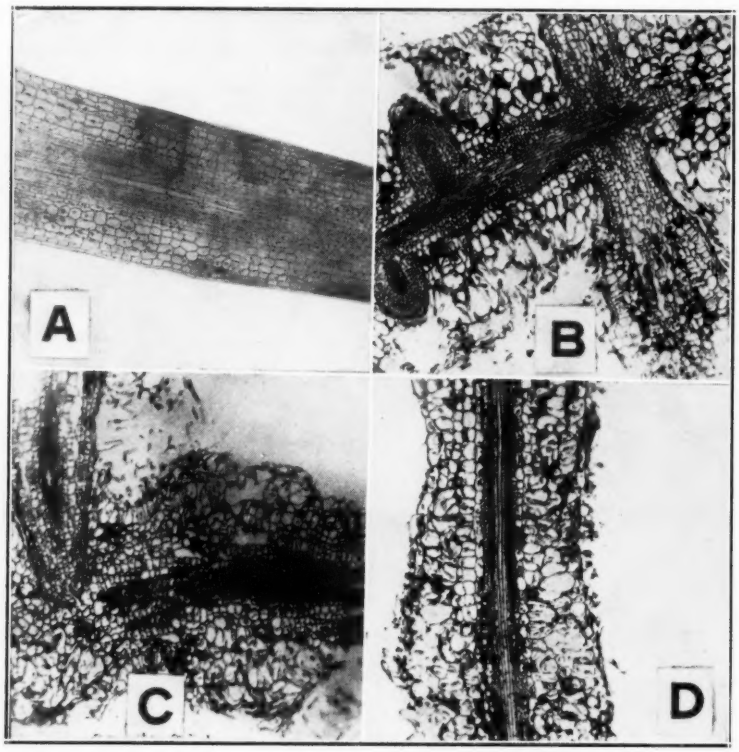

FIG. 4. Histological sections of tomato roots grown *in vitro* in the presence of indole-3-acetic acid. ×100. A. Normal untreated root section. B–D. Treated roots.

root primordia was observed. No effects comparable to those elicited by indole-3-acetic acid were seen when indole (200 to 0.1 mg. per liter) was added to the nutrient fluid.

The addition of acetic acid (10^{-2} to 10^{-8} molar) and of changes made by the addition of potassium hydroxide and hydrochloric acid in the pH of the nutrient solution (pH 0.95 to 11.5) were studied because acetic acid is part of the indole-3-acetic acid molecule, because it has been reported to cause plant tumors (17), and, also, because we have observed in various experi-

TABLE 2.—*The effect of indole-3-acetic acid and transfer to normal nutrient medium upon the growth and the number of secondary roots formed by excised tomato roots*

Number of root cultures per concentration	Concentration (mg./l.) of indole-3-acetic acid							
	5.0		0.5		0.05		0.025	
	L.	B.	L.	B.	L.	B.	L.	B.
10								
20—0.025	0	0	0	2	3	2	3	2
Transfer to normal nutrient medium								
Percentage survival	10		60		80		90	

ments that the addition of indole-3-acetic acid increases the acidity of the medium. The results with acetic acid were negative (20 root cultures). In the hydrogen-ion-concentration experiments (121 roots) no swellings were observed, but a few cultures showed increased root hair production, irregularity in shape, and excess development of root histogens; growth was maximal between pH 3.4 and 5.0. In table 3 are given the data from pH 3.0 to 5.7.

TABLE 3.—*The effect of hydrogen-ion concentration upon the growth and the number of secondary roots formed by excised tomato root tips*

Number of roots per H^+ concentration	Average pH of culture medium		Average growth[a]	
	Initial pH after sterilization	pH after culture period	L.	B.
5	3.0	.....	0	1
6	3.1	2.8	2	2
2	3.4	.....	29	6
5	3.5	.....	2	0
4	3.6	.....	39	4
6	4.1	5.3	45	4
6	4.2	4.0	54	4
5	4.4	4.7	41	3
6	4.8	.....	29	2
6	4.9	4.9	5	1
9	5.0	5.1	4	2
6	5.3	5.5	4	1
5	5.7	6.1	2	1

[a] Cultures were performed at different times of the year, at room temperature, and hence, conditions were different. Such variations affect the growth rate.

DISCUSSION

Although indole-3-acetic acid has been isolated from *Phytomonas tumefaciens* and many other bacteria and fungi (2, 12, 21), we have observed only slight tumor formation following employment of the extracted cultures of *P. tumefaciens*. The weak response noted may be attributed to the use of only 6.5 liters of broth, whereas from 140 liters Thimann (19) obtained only 1 mg. indole-3-acetic acid, and, furthermore, the difficulties of hormone extraction have been observed (11).

Brown and Gardner (3) first clearly demonstrated that structures arising from inoculation of phytohormones into whole plants resembled those produced by the crown-gall organism. The present study reports the production of galls in excised roots cultivated continuously *in vitro*.

The gross and microscopic responses of excised tomato roots to indole-3-acetic acid resembled those induced on intact and decapitated plants by this substance (3, 5, 7, 9, 12, 13), namely, gall and callus formation, inhibition of root elongation, hypertrophy, hyperplasia, root primordia initiation, necrosis, excess root-hair formation, and the presence of multinucleate cells. Similar responses of plants infected with *P. tumefaciens* have been recorded (12, 13, 18).

In this study it was not possible to demonstrate conclusively either stimulation or inhibition of tomato root growth with very dilute concentrations of

indole-3-acetic acid (10^{-10} to 10^{-14} molar). In these experiments a synthetic medium was employed to avoid possible introduction of indole-3-acetic acid in yeast extract. With similar root cultures it has been reported (16, 23) that indole-3-acetic acid was neither an essential nor growth-promoting substance; furthermore, tumor production was not mentioned. However, in carrot tissue cultures indole-3-acetic acid (0.000001 per cent) was reported (4) to promote growth, although at higher concentrations cell hypertrophy, root formation, meristematic activity, and callus formation were observed.

White and Braun (24) reported that bacteria-free secondary crown galls could be removed from plants, cultured *in vitro*, and when grafted back into plants crown gall (bacteria-free) resulted. We have observed that upon transfer into normal medium indole-3-acetic acid-root galls have no effect upon the growth of new tissue.

The possible slight activity of tryptophane observed in the present experiments may have resulted from the irritant effect of the toxic concentration employed, or to growth-hormone impurities present in the sample (21), as it is reported to be inactive in the Avena test (2). On the other hand, it is said to be active in the Pisum root formation test (21). Consistent with our observations, indole has not been observed to be an active substance (2, 21). Acetic acid and alteration of pH of medium failed to induce gall development. It has been stated that acetic acid is inactive in the pea test (21), that alteration in the pH of nutrient medium by this acid had no influence upon the growth of roots and root hairs (14), and that pH changes by hydrochloric acid did not affect inhibition of growth as indole-3-acetic acid did (6). Various acids, including acetic acid, have been reported to cause tumors (17), but acid effects, in certain cases, were believed (1) caused by conversion of phytohormone, already present in the plant, from an inactive to an active form.

In the present investigation overgrowths were shown to be incited only by concentrations of indole-3-acetic acid, which actually inhibited growth. The technic of plant tissue (or organ) culture has permitted a quantitative study of the relationship of growth and tumor formation to the concentration of phytohormone, and lends support to the hypothesis of the role of hyperauxony in the formation of galls (7, 10, 11, 12, 20, 21).

SUMMARY AND CONCLUSIONS

By means of ether extraction of culture broth in which *Phytomonas tumefaciens* had been grown indications were obtained that growth substance was produced during the course of its cultivation.

Excised root tips, growing in nutrient solution to which indole-3-acetic acid had been added, responded by the production of galls. Microscopically, the tumors were characterized by hypertrophy of the cortical and epidermal cells, by marked meristematic activity of the pericyclic cells resulting in abundant formation of root primordia, by some multiplication of cortical cells, by the occasional development of multinucleate cells in the pericycle

and cortex, by excess root-hair production with frequent swellings of the tips of the hairs, and by stimulation of callus formation at the cut ends of the roots. In the higher concentrations of the growth substance there was much fragmentation of the epidermal and cortical cells, and, in addition, the roots were thickened and of irregular shape.

Galls were formed only in those concentrations of indole-3-acetic acid that caused inhibition of growth.

Up to dilutions of about 7×10^{-9} moles per liter, indole-3-acetic acid inhibited root elongation, reduced the number of branches formed, and decreased the total dry weight. Concentrations of 10^{-10}, 10^{-12}, and 10^{-14} molar did not seem to accelerate the growth of isolated roots growing in continuous cultivation *in vitro*.

Five mg. indole-3-acetic acid per liter was the approximate lethal dose, killing about 90 per cent of the roots.

The chemical galls persisted after transfer to fresh nutrient solution, but all new growth in such media was normal.

The addition of tryptophane, indole, acetic acid, or variations in pH in control cultures failed to induce the formation of tumors.

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RESISTANCE TO MOSAIC VIRUS IN THE CUCUMBER[1]

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For the last 12 years various workers have attempted to develop mosaic-resistant cucumber varieties of high quality. This aim was not satisfactorily achieved. Moreover, the genetical mechanism involved in the manifestation of this disease seemed to be rather complex.

Porter (8) published the first paper on the reaction of cucumber to mosaic. In his breeding work he used the resistant variety Chinese Long, which he procured in China in 1925. According to Doolittle, *et al.* (2), breeding studies for mosaic and wilt resistance were initiated by the United States Horticultural Station at Beltsville, Maryland, in 1929. At this station both Chinese Long and Tokyo Long Green have been used as resistant parents.

Probably one of the greatest obstacles encountered in breeding cucumbers for resistance to mosaic is the apparent difficulty in differentiating various levels of resistance in the second and subsequent generations from crosses between resistant and susceptible parents. Thus, in the course of breeding operation for high quality and resistance, the latter character is progressively decreased.

The present preliminary report is concerned chiefly with the reaction of cucumbers to mosaic, the genetical mechanism involved in the manifestation of virus symptoms, and the criteria for detecting various degrees of resistance.

THE FIRST APPROACH TO THE PROBLEM

In April, 1940, F_2 progenies from the crosses China × Abbott and Cobb (A & C) and Shamrock × A & C were tested in the greenhouse for a study of the virus symptoms. Both China and Shamrock were known to be resistant to mosaic under field conditions. Various methods of inoculation were employed at the 3- to 5-leaf stage, the leaf-rubbing technique proving to be the most efficient. This method is similar to that described by Holmes (3).

All F_2 plants from these crosses showed mottling after inoculation, although there was a pronounced difference in the severity of these symptoms. In general, the F_2 progeny of China × A & C was more resistant than that of Shamrock × A & C. Some of the symptoms in the former cross were delayed for a considerable length of time and appeared on leaves located at different distances from the point of inoculation. Moreover, the delayed

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symptoms were not so severe and occasionally disappeared after a week or two from the date of their first appearance. No constant genetical ratio could be obtained from these experiments.

In order to determine the causes of such delay in the appearance of these symptoms, the same progenies and other resistant stocks were divided into groups and inoculated at the following leaf stages: cotyledon, first true-leaf, 2-leaf, 3-leaf, 5-leaf, 8-leaf, 11-leaf and 14-leaf. The results of these inoculation studies brought to light three important facts:

1. The delay in the appearance of symptoms is due primarily to genetical rather than to environmental factors.

2. There are 2 distinct types of symptoms of cucumber virus 1.

(a) Chlorosis—produced mainly on the cotyledons.

(b) Mottling—produced typically only on the true leaves.

3. All susceptible varieties are chlorotic at the cotyledon stage and all tolerant stocks fail to develop chlorosis on their cotyledons. Therefore, *the criterion for tolerance is the failure to produce chlorosis on the cotyledons following cotyledon inoculation.* As a result of these discoveries some changes in the breeding program were initiated in January, 1941, and some genetical data also were obtained.

EXPERIMENTAL MATERIAL

The following varieties were selected because of their practical and genetical interest:

1. *Chinese Long.* This inbred was obtained from R. H. Porter, of the Iowa Agricultural Experiment Station, and the variety has been described by Porter (8). This particular strain had been selfed previously for a few generations but inbreeding was continued for an additional four generations.

2. *China.* Obtained from Joseph Harris Seed Company, Rochester, New York. This variety is closely related to Chinese Long.

3. *Shamrock.* The variety was originally developed by R. H. Porter. Stocks were obtained from Porter, Vaughan's Seed Store, and A. E. Hutchins of the Minnesota Agricultural Experiment Station. One improved selection was developed at the Cornell University Agricultural Experiment Station from seed of the original Shamrock furnished by Dr. Porter.

4. *Abbott and Cobb (A & C) and Early Russian.* Seed obtained from J. C. Robinson, Waterloo, Nebraska. These are two of several susceptible varieties tested. Both were selfed for two generations before crosses were made.

The first three of the above varieties are known to be resistant to mosaic.

GREENHOUSE WORK

In January, 1941, crosses were made between the resistant parent Chinese Long inbred and the susceptible varieties Early Russian and A & C. The first-generation hybrids were tested and grown during the period March to

May, and F_2 seedlings were tested early in the spring of the same year before transfer to the field.

Method

Cucumber seeds in moist paper toweling were incubated at about 34° C. The seeds germinated after about 24 hours, and sprouting seedlings were planted directly in flats, 2-in. clay pots, or in $2 \times 2 \times 2\frac{1}{2}$-in. paper bands for transferring later to the field.

The plants were subjected to a 15-hour photoperiod (10 hours of daylight and 5 hours of artificial light) up to the set of the first fruit. The average air temperature was about 75° F. Extreme fluctuations of both temperature and light intensity were, however, the rule rather than the exception. During the incubation period, which lasted from 4 to 7 days, the temperature was maintained at approximately 85° F.

The original source of the virus was obtained from a diseased cucumber fruit. Young infected seedlings were used as inoculum. The virus symptoms on the true leaves were identical to those described by Doolittle (1) for cucumber virus 1.

The various stocks, F_1 and F_2 plants were all inoculated by the rubbing technique at the cotyledon stage. When inoculated, the cotyledons were well expanded, but the first true leaf was not yet fully developed. The tested plants were always inoculated together with a check series, comprising at least 100 susceptible plants. Because many inoculations were not very successful the data for the genetical analysis considered significant when the non-chlorotic plants of the check did not constitute much more than 10 per cent. The data from the F_2 were then adjusted.

EXPERIMENTAL RESULTS

Data on the reaction of the different stocks and hybrids to cucumber virus 1 are summarized in table 1. Inoculation was made at the cotyledon stage. Under greenhouse conditions all nonchlorotic stocks and hybrids, in contrast to the chlorotic varieties, were able to make growth beyond the seedling stage. The same varieties, stocks, and hybrids were inoculated and plants were subjected to subsequent infection during their life span in the field. It was possible then to distinguish at least 5 major groups in respect to the resistance of these plants. It is not yet established whether or not inoculation at the cotyledon stage only is sufficient for the demonstration of all these groups. However, in the case of the F_1 hybrids of the cross Chinese Long × A & C it was found that if cotyledons are inoculated and plants are protected from subsequent infection the symptoms may first appear on the third true leaf. It is possible, although no definite proof is available, that the virus invades all the resistant plants, and the presence or absence of symptoms depends on the age of the plant and the genotype involved. A brief description of the 5 major groups follows (Table 1 and Figs. 1 to 4).

1. *Symptomless.* Failure to develop symptoms during the life span. The inbred strain of Chinese Long, obtained from Dr. Porter, is probably

TABLE 1.—*Reaction of different cucumber stocks and hybrids to the virus. All were inoculated at the cotyledon stage and then seedlings in which virus infection was abundant were transferred to the field*

Variety or cross	Cotyledons	First true leaf	Subsequent leaves	Other symptoms and reactions	Field reaction
1. Chinese Long Inbred (Porter)	Nonchlorotic	Green	Green	.....	Symptomless
2. China (J. Harris Co.)	do	do	Mild mottling on 8–12 leaves. Eventually plants overcome these symptoms	.....	Highly resistant
3. Chinese Long × Early Russian (F_1) (Cornell)	do	do	The third true leaf is mottled. Plants eventually overcome the symptoms	.....	Resistant
4. (a) Shamrock R (Cornell)	do	Mottled	Mildly mottled or plants overcome symptoms	Stunting is not manifest	Tolerant to resistant
(b) Shamrock (Porter)	do	Mottled	Mildly mottled or plants overcome symptoms	Slight or no stunting	Tolerant
(c) Shamrock (Minn.)	do	Severely mottled	Mild to severe mottling	Stunted	Low tolerance
5. (a) Early Russian (J. C. Robinson)	Chlorotic	Severely mottled, curled downward	Severely mottled	Severe stunting. Plants remain at seedling size	Highly susceptible. Plants do not reach maturity
(b) Abbott & Cobb (J. C. Robinson)	do	do	do	do	do

homozygous for this character. Whether or not the virus actually invades this inbred was not determined.

2. *Highly Resistant.* Failure to develop symptoms up to about the 8- to 12-leaf stage. The variety China falls into this category. The plants recover from the relatively mild symptoms after a week or two from the date of their first appearance. Occasionally a few plants may show very mild

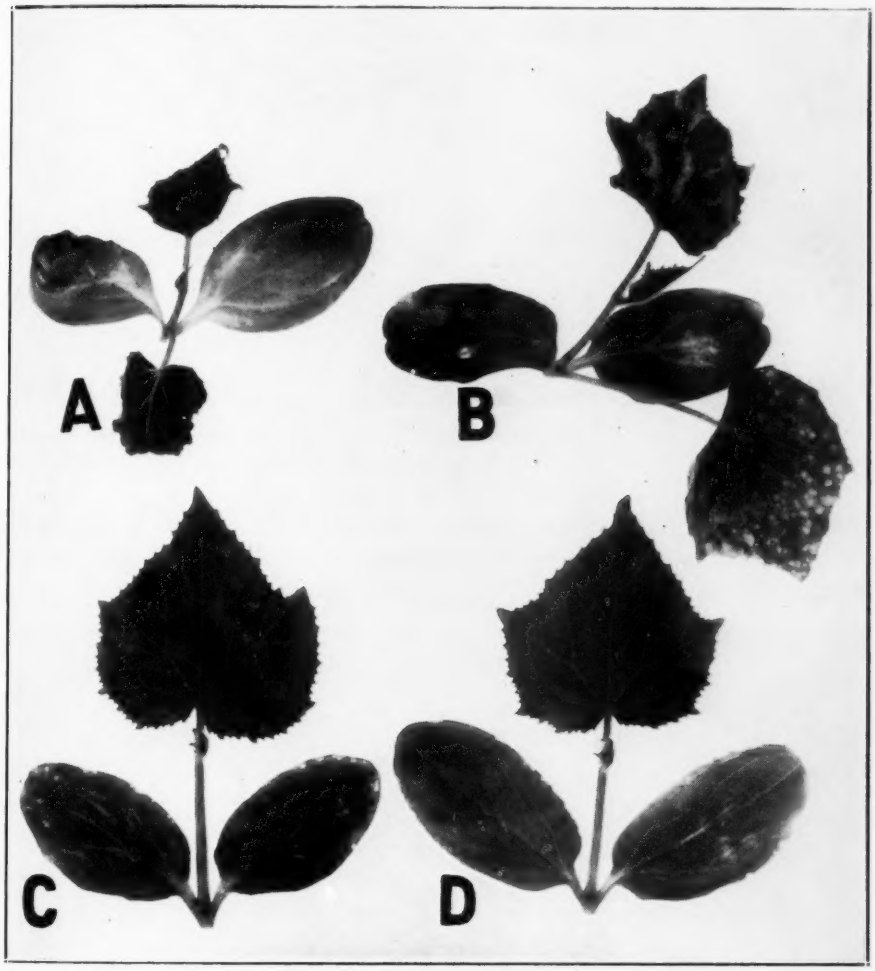

FIG. 1. Reaction of genetically different cucumber seedlings to cucumber virus 1. A. Susceptible. B. Tolerant. C. Resistant (F_1 of symptomless × susceptible). D. Symptomless (right). Under very favorable conditions the F_1 hybrids show mild symptoms on the first or second true leaves.

symptoms at the 1- or 5-leaf stage. This may be due to the heterozygosity of the variety.

3. *Resistant.* Failure to develop symptoms up to about the 3-leaf stage. All hybrids of *symptomless* × *susceptible* may fall into this category. The plants recover from the mild symptoms but new symptoms may appear again

FIG. 2. Reaction of the resistant variety China (left) and the susceptible variety A & C (right) to cucumber virus 1 under field conditions. Both varieties were inoculated at cotyledon stage.

and subsequently disappear. Under very favorable conditions for virus multiplication mild symptoms may appear on the first true leaf of these hybrids.

FIG. 3. Resistance to cucumber virus 1 is dominant from a practical standpoint. The two rows with luxuriant plant growth were planted to hybrids symptomless × susceptible. At the left side note the comparative response of tolerant and susceptible stocks. All plants were inoculated at cotyledon stage.

4. *Tolerant.* Failure to develop chlorosis on the cotyledons. Symptoms on first true leaf may be mild or severe, at least for a given period of time. The data in table 1 indicate that all strains of Shamrock have these characteristics. However, they differ in their ability to overcome the symptoms and to reach maturity without great loss in yield. The most tolerant strains of this group do not show any symptoms on the fruit (Fig. 4) and in this respect they possess this important characteristic of the resistant groups.

5. *Susceptible.* Ability to develop chlorosis on the cotyledons. All commercial varieties produce chlorosis following cotyledon inoculation. Chlorosis is followed by severe mottling and stunting of the plants. Under greenhouse conditions the plants may be killed within a short period or

FIG. 4. A tolerant selection (F_5) from a cross Shamrock × A & C. Although some mottling is present on the leaves the plant is not very stunted and fruit is not impaired. This is an excellent slicing type. (See seedling reaction of the same stock in figure 1.)

remain stunted at the seedling stage for a long time, becoming rather "woody" in appearance and never reaching maturity. Also in the field these stunted plants do not reach maturity but they do not die as do vigorously growing plants which become infected at a later stage. If susceptible plants are infected at maturity the fruits may show mottling and are unmarketable (1).

THE GENETICAL MECHANISM

Very meager data have been published on the genetical control of plant viruses. Holmes (4, 5) described four interesting types of responses to tobacco virus 1 (distorting strain) in *Capsicum frutescens*, which he attributed to a series of allelomorphic genes. Nolla (7) found that in tobacco inheritance of reaction to tobacco mosaic is controlled by duplicate genes and that resistant plants have the double recessive genes. Holmes (6) concluded that in *Nicotiana* the ability to produce necrotic primary lesions is

due to a single dominant gene, and the ability to produce systematic chlorosis is due to the recessive allele. In this case the conclusion was based on data obtained from a cross between *Nicotiana digluta* (necrotic type) and *Nicotiana tabacum* (chlorotic type). The F_1 has two sets of *tabacum* chromosomes which synapse and one independent set of *glutinosa* chromosomes (24TT + 12G). From such breeding material one could not expect to obtain a Mendelian ratio or to prove genetically the allelomorphic nature of the characters necrosis and chlorosis.

Porter (8) suggested that resistance to cucumber mosaic in the cucumber is due to one or a few recessive genes. Although not valid, such a conclusion may be made if data be obtained from growing F_2 seedlings only.

In order to gain a satisfactory understanding of the genetical mechanism involved in the manifestation of cucumber virus 1, it is essential to consider 2 distinct developmental phases: 1. *The cotyledon phase,* in which only the reaction of the cotyledons is considered. 2. *The composite true-leaf phase,* in which the response of any given leaf stage is referred to.

It was definitely established that resistance, as indicated by the failure to produce chlorosis, is dominant at the cotyledon stage. First generation hybrids of symptomless × susceptible, resistant × susceptible, and tolerant × susceptible react similarly in this respect.

As these hybrids reach the true-leaf stage they invariably show mottling on their leaves. In these hybrids, however, the adverse effect of the virus varies from no pronounced effect to a relatively marked stunting, depending on the genotypes involved. The hybrids of Symptomless × Susceptible manifest very mild symptoms, which soon disappear and are not affected to any appreciable degree by the invasion of the virus. On the other hand, hybrids of tolerant × susceptible are somewhat stunted at early stages, although eventually they do overcome this condition.

A summary of the data on the reaction of F_2 seedlings at the cotyledon stage is presented in table 2. The observed ratio between nonchlorotic and chlorotic plants is in good agreement with the theoretical 27 : 37 ratio, indicating the action of 3 complementary genes. Only 48 plants of the backcross F_1 (symptomless × susceptible) × *susceptible* were inoculated at the cotyledon stage, together with a check series of 61 seedlings. The check series became 100 per cent chlorotic, while 6 plants of the backcross population of 48 were nonchlorotic. Although this is a perfect 1 : 7 ratio, as would be expected on the basis of 3 complementary genes, the size of the population is rather small.

Thus, at the cotyledon stage, the F_2 population is divided into chlorotic and nonchlorotic groups. As this F_2 population reaches the true-leaf stage the ratio 27 "resistant" to 37 "susceptible" is constantly changing during development. Of 523 F_2 plants only 3 were suspected to be symptomless through their life span. It is apparent that any attempt to secure a Mendelian ratio from growing F_2 plants is futile. Not only the age of the plant but temperature and other environmental factors have a pronounced effect

upon inhibition or stimulation of the appearance of symptoms during development. Moreover, some plants seem to overcome the previous symptoms and will, therefore, be classified erroneously as symptomless. It appears that in order to secure reliable data relative to the total genes involved in the manifestation of mosaic symptoms, it is essential to keep weekly records on each F_2 plant at least to the stage in which symptoms first appear.

The fact that only 3 symptomless plants were recovered out of 523 F_2 plants may suggest a 1:63 ratio to correspond with the initial 27:37 ratio at the cotyledon stage. This conclusion, however, cannot be justified, because it is possible to isolate several homozygous races which vary in their degree of resistance. On the basis of 3 complementary genes there can be only 1 homozygote for resistance; therefore, it is necessary to assume the presence of 1 or several gene modifiers in addition to the 3 basic genes.

TABLE 2.—*Segregating F_2 population resulting from a cross between Chinese Long inbred, resistant to virus, and Early Russian inbred, susceptible to virus. All seedlings including the susceptible check plants were inoculated at the cotyledon stage. Only the characters nonchlorotic vs. chlorotic cotyledons are considered in the genetical classification. Total number of F_2 plants, 812*

Notation of cross	Cotyledon	Observed number of plants	Susceptible check plants	Adjusted numbers (A)	Calculated numbers (27:37) (C)	Difference (C − A)	D/P.E.
CE1	Nonchlorotic	176	18	152	165	13	2.0
	Chlorotic	216	164	240	227		
CE2	Nonchlorotic	188	7	171	177	6	0.9
	Chlorotic	232	93	249	243		
Total 812	Nonchlorotic	364	25	320	343	23	2.4
	Chlorotic	448	257	492	469		

These modifiers, it is assumed, are chiefly effective only after the cotyledon stage. The homozygous strains that vary in degree of resistance have either the 3 dominant genes and all the recessive modifiers, or the 3 dominant genes and 1 or more dominant modifiers. The true symptomless plant is assumed to be homozygous to the 3 basic dominant genes and to the maximum dominant modifiers present. The true ratio between symptomless and symptomatic plants is not 1:63, but at least 1:255 or 1:1033.

The F_2 chlorotic group also is not uniform in its reaction and, as a whole, is less susceptible to the virus than is the check; in fact, very rarely is a chlorotic plant found in this group that is so severely affected by the virus as is the check plant. This evidence further substantiates the thesis of multiple factors as applied to this case. The true susceptible plant may be assumed to be homozygous to the 3 basic recessive genes and to the maximum recessive modifiers.

Thus it appears that the genetical mechanism involved in the manifesta-

tion of virus symptoms in the cucumber follows a developmental pattern. At the cotyledon stage 3 complementary genes are responsible for the ability or the failure to produce chlorosis. As the F_2 population reaches the true-leaf phase, the initial genetical ratio is constantly changing during development. At this phase several gene modifiers, in addition to the 3 basic genes, also play a role in the manifestation of virus symptoms. As a result the frequency of symptomless plants is exceedingly low.

Criteria for Measuring Resistance

In cases where resistance and susceptibility cannot be regarded as distinct qualitative characters, a general method for detecting degrees of resistance is of paramount practical importance. Nolla (7), working with tobacco, determined degrees of resistance to tobacco virus by the relative number of local lesions produced by the infected plants on *Nicotiana glutinosa*, *Nicotiana repanda*, and *Nicotiana glutinosa* × *Nicotiana tabacum*. By this method he discovered that the symptomless plants carried the active virus also.

In the case of the cucumber the above procedure is not possible, because cucumber virus 1 will not produce local lesions on *Nicotiana glutinosa* or other known plants. However, we do have a reliable and very simple method for detecting degrees of resistance to mosaic in the cucumber. The first requirement of this method is *cotyledon inoculation*. The criterion for tolerance is the failure to produce chlorosis on the cotyledons. All tested plants that are non-chlorotic are subject to further observation during seedling development. The degree of tolerance is determined by the relative distance from the inoculated cotyledons to the true leaf on which symptoms appear. The greater the distance the greater is the resistance. The above statements should not be considered as a mathematical formula. They do serve, however, as a guide for the breeder. Temperature and other environmental factors have great influence on the appearance of symptoms, and therefore in making decisions one should also be guided by the severity or mildness of the symptoms and by the rapidity with which plants overcome them.

SUGGESTIONS TO PRACTICAL BREEDERS

In order to produce symptomless varieties of cucumbers it is essential to use a resistant parent of the Chinese Long type. On the basis of an F_2 ratio of 1 : 63, not less than 460 plants should be grown in order to obtain, with a reasonable degree of assurance, 1 symptomless plant. However, as has been shown in this paper, more genes are involved in the inheritance of resistance to mosaic and therefore a much larger F_2 population should be grown. For example, a minimum of 7,450 plants must be grown if five genes are involved and 119,270 if seven genes are responsible for the inheritance of resistance to this disease. The task of isolating symptomless plants is not so difficult as it may appear. At the cotyledon stage it is possible to discard about 57 per cent of the F_2 seedlings after inoculation. Within a

period of six or seven weeks more than 90 per cent of the total F_2 population can be eliminated. The seedlings can be grown in the greenhouse or in the field. In the latter case they should be planted very thickly, all symptomatic plants being discarded at weekly intervals. Several thousand plants can be handled in this way without much difficulty. Repeated crosses of symptomless selections with the commercial types will result in isolation of desirable symptomless varieties. The two great advantages of this method are:

1. A symptomless variety will prove to be more desirable in the long run from an economical point of view. Tolerant varieties are more apt to furnish the basic material for virus mutation and source of inoculum.

2. The breeder can be sure that his selections are always homozygous for resistance and will breed true for this character. Other breeding methods can be used, the important consideration being that each selfed generation must be subjected to cotyledon inoculation and selected plants preferably should be symptomless up to the 3-leaf stage. By this inoculation method high resistance can be maintained during the breeding program.

SUMMARY

The genetical control of virus symptoms in the cucumber and criteria for measuring resistance are discussed in this preliminary report.

In order to understand the genetical mechanism involved it is essential to distinguish between two developmental phases, (1) the cotyledon stage, and (2) the composite true-leaf stage.

1. Three complementary genes apparently control the ability or the failure to produce chlorosis at the cotyledon stage, the genetical ratio in F_2 being 27 nonchlorotic to 37 chlorotic.

2. The ratio of 27 : 37 is constantly changing at the composite true-leaf stage. At this stage several gene modifiers also take part in the genetical control of virus symptoms. Thus, the frequency of symptomless plants is very low.

3. The presence or absence of chlorosis on the cotyledons determines whether the tested plant is susceptible or tolerant to mosaic.

4. The degree of tolerance can be determined by the severity or mildness of symptoms and by the relative distance from the inoculated cotyledons to the true leaf on which symptoms first appear. The greater the distance the greater is the resistance.

5. All resistant stocks possess the three basic dominant genes. They vary among themselves in the relative number of dominant modifiers.

Some suggestions to plant breeders are included in this paper.

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EXPERIMENTAL AUTOECISM AND OTHER BIOLOGICAL STUDIES OF A GALL-FORMING PERIDERMIUM ON NORTHERN HARD PINES[1]

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INTRODUCTION

In view of the relatively meagre extant knowledge regarding aeciospores from pine, capable of repeating infection on pine, Arthur[3] suggested to Snell the possible autoecism of certain forest-tree rusts. In June, 1932, Snell collected aecia of a bark rust on *Pinus banksiana* Lam. at Schuyler Falls, New York. Later, the writer collected aecia from galls on *P. banksiana* (Fig. 1, D) near Schuyler Falls, and aecia from galls on *Pinus rigida* Mill. at Monument Beach, Massachusetts.

All of the material collected was apparently the heteroecious gall-forming *Peridermium, P. cerebrum* Peck,[4] since the uredial and telial stages of *Cronartium quercuum* (Berk.) Miyabe were found by Snell[5] in both localities on nearby oaks.

No reports of autoecious aeciospores on *Pinus banksiana* capable of infecting *P. banksiana* or other pines, or the successful inoculation of *P. banksiana* with repeating aeciospores from other pines are to be found in the literature. The present study concerns the successful inoculation of *Pinus sylvestris, P. banksiana,* and *P. rigida* with aeciospores from *P. banksiana* and *P. rigida,*[6,7] together with other observations of certain pathological phenomena, physiological disturbances, and resistance to the spread of the fungus in infected trees.

[1] Part of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Brown University, June, 1935, together with additional data obtained in experiments at the Massachusetts Agricultural Experiment Station. Massachusetts Agricultural Experiment Station Contribution No. 418.

[2] The writer is indebted to Professor Walter H. Snell of Brown University, under whose direction investigations reported here were undertaken, for advice during the progress of the work and criticism of the manuscript. Grateful acknowledgment for criticism of the manuscript is also made to Dr. Alma M. Waterman, U. S. Division of Forest Pathology; Professor J. S. Boyce, Yale University; and Professor A. Vincent Osmun, Massachusetts State College.

[3] Personal communication to Professor Walter H. Snell of Brown University from Professor Joseph C. Arthur of Purdue University.

[4] Arthur (6) lists *Peridermium cerebrum* Peck (in parentheses) and *Cronartium cerebrum* Hedgc. and Long among the 11 synonyms for *Cronartium quercuum.* The principal interest of this paper is not in the nomenclature of the rust, however, nor in its taxonomic position, and the designation of the rust involved as *Cronartium quercuum* is used by the writer without overlooking the fact that later investigations may necessitate a change of specific epithet.

[5] Professor Walter H. Snell, personal communication.

[6] George G. Hedgcock of the U. S. Department of Agriculture stated in a letter that he had tried numerous inoculations on pines with aeciospores of both *Peridermium cerebrum* and *Peridermium fusiforme* and had never observed the production of galls.

[7] In May, 1932, just previous to the experiments described in this paper, Professor Snell inoculated pines with aeciospores of *Cronartium quercuum* from *Pinus rigida* collected in Massachusetts. His experiments have shown no evidence of repeating aeciospores.

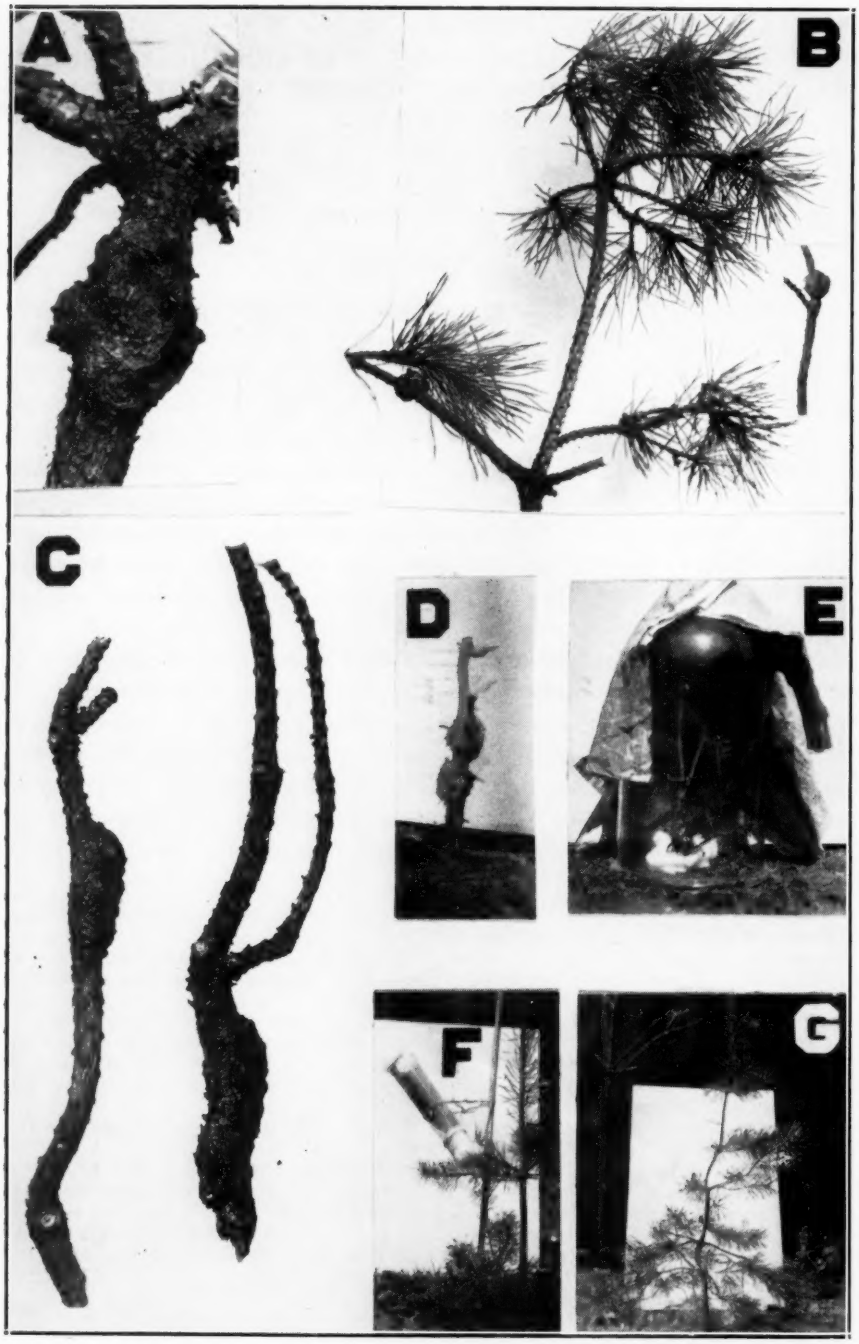

FIG. 1. Galls produced on *Pinus sylvestris* (A, B, C) after inoculation with aeciospores collected from fruiting galls shown in D (galls about 2½ inches in diameter). A and C, approximately natural size; B, somewhat reduced. E. Shaded bell jar used as inoculation chamber. F. Celluloid cylinder used as inoculation chamber. G. 7-year-old check tree of *Pinus sylvestris*.

REVIEW OF LITERATURE

There are few reports in the literature presenting conclusive evidence regarding the autoecism of aeciospores in rusts of forest trees, but these, together with certain of the more numerous reports of investigations on rusts in general, are of considerable interest in connection with the present problem.

Concerning the simplification of the life-cycle of rusts, Arthur (5, p. 184) states "There is a general impression among uredinologists that the length of cycle shows a tendency to shorten in proceeding from the equator toward colder regions of the north." He cites work by Magnus, Ivanov, Morgan-thaler, and Fischer to support this statement and adds "The same proposition is further strengthened in tracing the distribution of the microcyclic rusts." Reports of the heteroecious species of the genus *Cronartium*[8] in which aecia may produce repeating spores include a *Peridermium* on *Pinus attenuata* Lemm., *P. contorta* Dougl., *P. jeffreyi* Balf., *P. ponderosa* Dougl., and *P. radiata* Don. in the western United States (26, 27), and *Peridermium pini* on *Pinus sylvestris* in central Europe (14, 22). Spaulding (33) states: "In 1913 Meinecke [26] made successful inoculations with aeciospores of '*Peridermium harknessii* J. P. Moore' upon *Pinus radiata* under controlled conditions. Later (1920) he changed the name of the fungus to *Peridermium cerebrum* and reported that he had successfully inoculated *Pinus radiata* with aeciospores from *P. radiata* and from *P. attenuata;* and *P. muricata* with aeciospores of *Peridermium cerebrum* from *Pinus radiata.*" In 1929 Meinecke expressed doubt concerning the assignment of the name *Peridermium cerebrum* to the rust referred to in his inoculations and proposed the name *Peridermium cerebroides* (?). He then assigned the name *P. Harknessii* (*Cronartium harknessii* nov. comb.) to the other California pine gall rust, apparently confined to the mountains (28). Arthur (6) refers "*Peridermium harknessii*" (*Cronartium harknessii* Meinecke), *Peridermium filamentosum* Hedgc., and *Peridermium stalactiforme* A. and K. to *Cronartium coleosporioides* and states that the three forms may be treated as distinct species or as varieties under the names *Cronartium coleosporioides harknessii, C. c. filamentosum, C. c. stalactiforme.* Arthur also states: "The form on *Pinus radiata* and *P. attenuata,* sometimes referred to *Cronartium quercuum,* has been shown by Meinecke [26, 27] in many cultures to form repeating aecia on *P. radiata.* Similar cultures were also made by Meinecke [28] with aecia from *Pinus contorta* and *P. sabiniana* sown on *P. contorta, P. radiata, P. Jeffreyi,* and *P. Coulteri.*" The report by Haack (14) that he obtained abundant infection from inoculations of *Pinus sylvestris* with aeciospores of *Peridermium pini* from the same host is discredited by Spaulding (33) because of the conditions under which the experiments were conducted. Under controlled conditions, however, young twigs of *Pinus sylvestris* with and without wounds were successfully inoculated with aeciospores of *Peridermium pini* by Klebahn (22), although

[8] No heteroecious species of any other genus are known to produce repeating spores in the aecial stage (6).

Liro (24) eleven years earlier had reported that no infections resulted from 169 inoculations of *Pinus sylvestris* with aeciospores of *Peridermium pini*. *Pinus ponderosa* var. *scopulorum* Englem., *P. contorta*, *P. sabiniana* Dougl., *P. caribaea* Morelet, *P. mariana* Du Roi, *P. pinea* L., and *P. halepensis* Mill. were successfully inoculated with aeciospores of "*Peridermium Harknessii*" from *P. contorta;* and *P. ponderosa* and *P. virginiana* Mill. were also successfully inoculated with aeciospores of "*Peridermium Harknessii*" from *Pinus ponderosa* by Hedgcock and Hunt (15). Also, a bark rust, "Woodgate Rust," on *Pinus sylvestris*, in which no alternate form is known, produces a gall somewhat like that of *Peridermium cerebrum*.

Spaulding (33) reports that the possibility of autoecism of *Cronartium ribicola* Fisch. (*Peridermium strobi* Kleb.) was considered as early as 1913, "but no evidence of the spread of the fungus directly from pine to pine was found." He cites work by Klebahn, Hennings, and himself in which negative results were consistently obtained.

Galloway (12) reports a microcyclic rust, *Gallowaya pinicola* Arth.,[9] which occurs on the leaves of *Pinus virginiana* throughout the range of the host in the Allegheny Mts. from Maryland to Tennessee. This rust is also reported to have occurred in western Siberia on *P. cembra* L. (6). At one time the correlated macrocyclic species appeared to be *Coleosporium helianthi* (Schw.) Arth. (5), but recently Arthur (6) stated "The correlation of this microcyclic species with a particular macrocyclic form is somewhat uncertain, but it seems to accord well with *Coleosporium inconspicuum* (Long) Hedge. & Long (*Peridermium inconspicuum* Long), less so with *C. helianthi.*"

In studies of host resistance, Palladin (29) concluded from his work with cereals grown in silicic-acid-deficient solutions that silica in cell walls may have an important part in protecting plants against rust infection. Klebahn (21) considers the difficulty of obtaining successful inoculations with rust fungi in old leaves to be a result of the greater resistance of the cell walls of the epidermis. Arthur (3, 4), Sappin-Trouffy (31), Cobb (9), and Hursh (17) have found that the morphological nature of the host may qualify the development of the mycelia in the host plants. On the other hand extensive investigations by Ward (41), Eriksson and Henning (10), Biffen (7), Vavilov (38, 39), Garber (13), Allen (2), Hursh (17), and Zimmermann (43) have frequently demonstrated that morphological conditions cannot always explain resistance. Hutchinson (18) suggests that resistance to rust infection may be morphological or physiological or a combination of these types.

Various physiological phenomena have been investigated by many observers in connection with infection by rusts. Photosynthesis, transpiration, respiration, and tests of cell contents have been subjects of numerous studies. According to reports, except in the case of "green islands" described by Mains (25) and Rice (30), both gametophytic and sporophytic rust mycelia

[9] Arthur refers *Gallowaya pinicola*, *Gallowaya pini* Arth., and *Coleosporium pini* Gall. to *Coleosporium pinicola* n. comb.

in host plants cause a decrease in the amount of chlorophyll in the cells and may limit photosynthesis to such an extent as to prevent further development by the parasite. Numerous reports in the literature indicate that transpiration is considerably increased in rust-infected plants, although there are few accurate data available on this point. The various reports concerning the effect of rust infection on respiration agree uniformly in showing a greater intensity of respiration following infection. Except for certain records, which may require special explanation, most observers state that there is a definite relation between the relatively large quantity of starch present during the early growth of the fungus and the diminution of starch as the fungus becomes established. In general, reports agree that all degrees of chlorophyll loss and plastid destruction may result in rust-infested plants. Evidence concerning calcium oxalate in such plants apparently is conflicting, since both increased and decreased amounts are reported (11, 34, 40). Fatty or resinous substances may be present in abundance in rust-infested conifers owing to the increased number of resin ducts, according to Adams (1). Likewise, byproducts resulting from a disturbance of normal conditions in plants may result in maize and sorghum. Rice (30) indicates that various color-producing substances, notably anthocyanin, may occur when these plants are infected.

GERMINATION OF AECIOSPORES FROM COLLECTED MATERIAL

Spores from all galls collected in 1932 and 1934 for the inoculations just described germinated readily for a period of approximately four weeks. After that time germination was erratic. Cooling with ice was found to stimulate germination. Each of the germinated spores was observed to produce from 1 to 3 germ tubes, which became somewhat branched within 12 hours. Under favorable conditions approximately 90 per cent of the spores germinated in 12-hour periods. The optimum temperature for germination in distilled-water drop cultures was 10 to 12° C. Germination did not occur above 20° C. or below 5° C. No spores were observed to germinate later than 10 weeks after collection. Reports on the longevity of spores of *Cronartium ribicola* have been rather completely reviewed by Spaulding (33).

In the sectioned material prepared in the present studies, the cells of the mycelium giving rise to aecia were uninucleate. The spores produced by the aecia were binucleate, and upon germination were observed to form germ tubes, but never basidia (promycelia). The germ tubes of the rust spores under investigation differ from those of the aeciospores of *Cronartium ribicola* in that those of the former are fewer in number and branch less. Otherwise, the present observations and results are similar to those reported for the germination of aeciospores of *C. ribicola* and the germination of spores in Meinecke's experiments on repeating pine rusts. Also, the spores of the woodgate rust from galls on *Pinus sylvestris* have been observed to germinate with a germ tube (True (37)). Therefore, from the results of

the germination studies, it would appear that the spores as reported by Meinecke (28), the spores of the woodgate rust from galls on *P. sylvestris*, and the spores concerned in the present study were aeciospores in the proper sense of the term, and not different from the aeciospores of *C. ribicola* and other heteroecious forest-tree rusts, except that the germ tubes of the former will reinfect the aecial host.

EXPERIMENTAL METHODS

In June, 1932, experiments were begun with 12 trees of *Pinus sylvestris* and 6 trees each of *P. banksiana* and *P. rigida*, exclusive of checks; and in June, 1934, experiments were begun with 20 trees of *P. banksiana* and 12 trees each of *P. sylvestris* and *P. rigida*, exclusive of checks. All trees were certified, inspected nursery stock less than 10 years old and were free from physical defects. The pines were inoculated out-of-doors, and no rust or other evidence of infection was apparent prior to the inoculation of the trees. All practicable precautions were taken to limit outside factors.

In the 1932 experiments, the trees were inoculated with aeciospores collected by Snell from *Pinus banksiana* in a forest of natural growth near Schuyler Falls, N. Y. In the 1934 experiments the trees were inoculated with aeciospores collected by the writer in the New York location and also in Massachusetts.

The technique employed in the inoculation of the pines included operations similar to the modification of Hubert's technique employed by York (42) in his inoculations with the woodgate rust fungus and Meinecke's (28) technique in his experiments with repeating pine rusts, and the modifications of Hunt's (16) iceless-refrigerator method described by Snell and Gravatt (32). The details are given here only for the use of celluloid cylinders shaded from direct sunlight.

The trees were inoculated immediately after sun-down by depositing fresh, ripe aeciospores with a sterile camel-hair brush upon wet unwounded and wounded bark of growth of the current season and previous years. Wet cotton wicks were wrapped at the lower limit of the inoculations if the trees were small enough to be covered with bell jars (Fig. 1, E). On larger trees celluloid cylinders (Fig. 1, F) with wet cotton plugs at either end were used to cover the inoculations. All cotton plugs and wicks were kept wet and the inoculated trees were kept in a moist atmosphere, shaded from direct sunlight for four days following inoculation. Some of the checks were given exactly the same treatment as inoculated trees except for the application of the aeciospores, while some checks were left untouched (Fig. 1, G). Most of the material for sectioning was collected from inoculated and uninoculated trees at intervals varying from 24 hours to almost three years during the course of the experiments.

In the preparation of tissues for sectioning, the following killing and fixing solutions (8, 23, 35) were used: osmic acid (2 per cent aqueous solution), picric acid in concentrated aqueous solution, Formalin alcohol (5 parts

Formalin to 100 parts 70 per cent alcohol), Flemming's mixture (an aqueous solution of chromic acid, 0.25 per cent, containing 0.1 per cent of osmic acid and 0.1 per cent acetic acid), Carnoy's solution and Schaffner's chromo-acetic acid solution. The last-mentioned solution was the most successful. Some material was imbedded in paraffin following Zirkle's (44) butyl-alcohol method and later sectioned on the rotary microtome in order to obtain serial sections. Other material was imbedded in nitrocellulose, as outlined by Jeffrey (19, 20), and cut on the sliding microtome. Jeffrey's method was particularly successful in the preparation of gall tissue for sectioning. Free-hand sections were cut directly from freshly collected material and some sections were cut by the ether freezing-microtome from material boiled in water. Transverse, radial and tangential sections 10–20 μ thick were cut in the various ways mentioned.

Three different combination stains were tried on the sections: Delafield's haematoxylin and erythrosin; Flemming's safranin, gentian violet, and orange G; and the orseillin and anilin-blue stain described by Strasburger (36). The stain combinations of Delafield and Flemming proved very satisfactory for the study of uninfected tissue. For the study of mycelium in host tissue the orseillin and anilin-blue combination stain was most helpful.

In connection with the study of normal and infected tissue, microchemical tests were made on freshly cut freehand sections for the identification of cellulose, cutin, lignin, suberin, pectin, starch, fats, gums, tannin, and resin. The tests recommended by Kraemer (23) were followed.

FIELD OBSERVATIONS

All of the inoculated trees of *Pinus sylvestris* and *P. banksiana* and some of *P. rigida* have shown varying responses to infection, including: bark discoloration, gall formation, leaf casting, witches'-brooms, dwarfing, and necrosis. A few of the inoculated *P. rigida* trees developed no symptoms of infection and none of the checks at any time appeared to be infected. Observations showed infection to be limited to the growth of the season of the inoculations. These inoculations resulted in the production of mycelium in all of the species of pine tested, but galls were formed only on *P. sylvestris* inoculated with aeciospores from *P. banksiana*. Seven galls were formed on one 5-year-old tree of *P. sylvestris*. Certain of the galls resulted in the formation of witches' brooms. Numerous investigations of witches' brooms associated with rust infection have been previously reported (5, 6, 28). The galls (Fig. 1, A, B, C; Fig. 2, B) were not uniformly characteristic of any particular rust. Sometimes they were elongated swellings along the stem. At other times they showed indications of forming almost perfect pyriform structures. No aecia or pycnia appeared on the galls, although mycelium was abundant throughout the gall tissues. Arthur (6) indicated that pycnia were usually not found associated with galls in autoecious rusts, although True (37) reported them in woodgate rust. At no time did the young galls appear similar to the typical young galls found in woodgate

rust. It should be noted here that reports on the latter rust indicate that its aeciospores will not infect *P. banksiana.*

On all species of pine inoculated with aeciospores from all collections there were observed numerous cases of slight roughening, swelling, or discoloring of the bark, which did not result in the formation of galls. Similar phenomena for other rusts are described by Hutchinson (18) and others. Some cases were observed where the leaders of inoculated pines were very heavily infected and discoloration of the bark was noticeable. In such cases leaf casting and death of the leader, or in the case of the very young trees, of the entire tree, were not uncommon. In still other cases dwarfing resulted. Although the death of cells, organs, or entire plants is comparatively rare in connection with rusts as a group, because of their almost perfect adaption as obligate parasites, necrosis does occur in certain cases. For example, the death of young trees of *Pinus sylvestris* and *Pinus montana* Mill. results from attack by a rust commonly called the "pine twister" so named because the mycelium of the aecial stage causes the shoots to twist about the point of attack. Also, dwarfing or death of trees commonly results when white pines under 20 years of age are infected with *Cronartium ribicola.*

HISTOLOGICAL STUDIES

Histological studies of noninfected tissues of the various pines concerned in the present study showed no important cytological differences between the trees that developed galls, the trees in which rust infection resulted in severe defoliation or death, the trees showing no effect from inoculation, and the checks. Likewise, microchemical tests on fresh sections from the various trees showed no significant differences in the stored food material or in the composition of the cell walls. Certain quantitative variations in fat and tannin were observed, but numerous investigations in addition to the present study indicate that these substances may vary considerably in plants, even in different twigs of the same plant.

In a study of the mycelium in the bark of inoculated pines, the hyphae were always intercellular and followed along the rays past the cambium and into the xylem. In this latter tissue the hyphae were confined chiefly to the ray cells. In tangential sections, the cortex and especially the phloem showed abundant mycelium in strands. Groups of phloem parenchyma cells often were surrounded by strands of hyphae. In both the phloem and xylem rays, hyphae may completely fill the enlarged intercellular spaces, which have resulted from the advancement of the mycelium.

Haustoria (Fig. 2, A) are apparently always unicellular. They were seldom found in young hyphae, but in old infections were observed in all types of host cells, including resin cells and the lumens of the tracheids. The formation of haustoria is thought to indicate a change in the nutritional relationship (5, p. 230) of the parasite and host, but the exact relationship is not known. The haustoria apparently have the power to pierce the cell walls at any point, but no further evidence relative to their function was

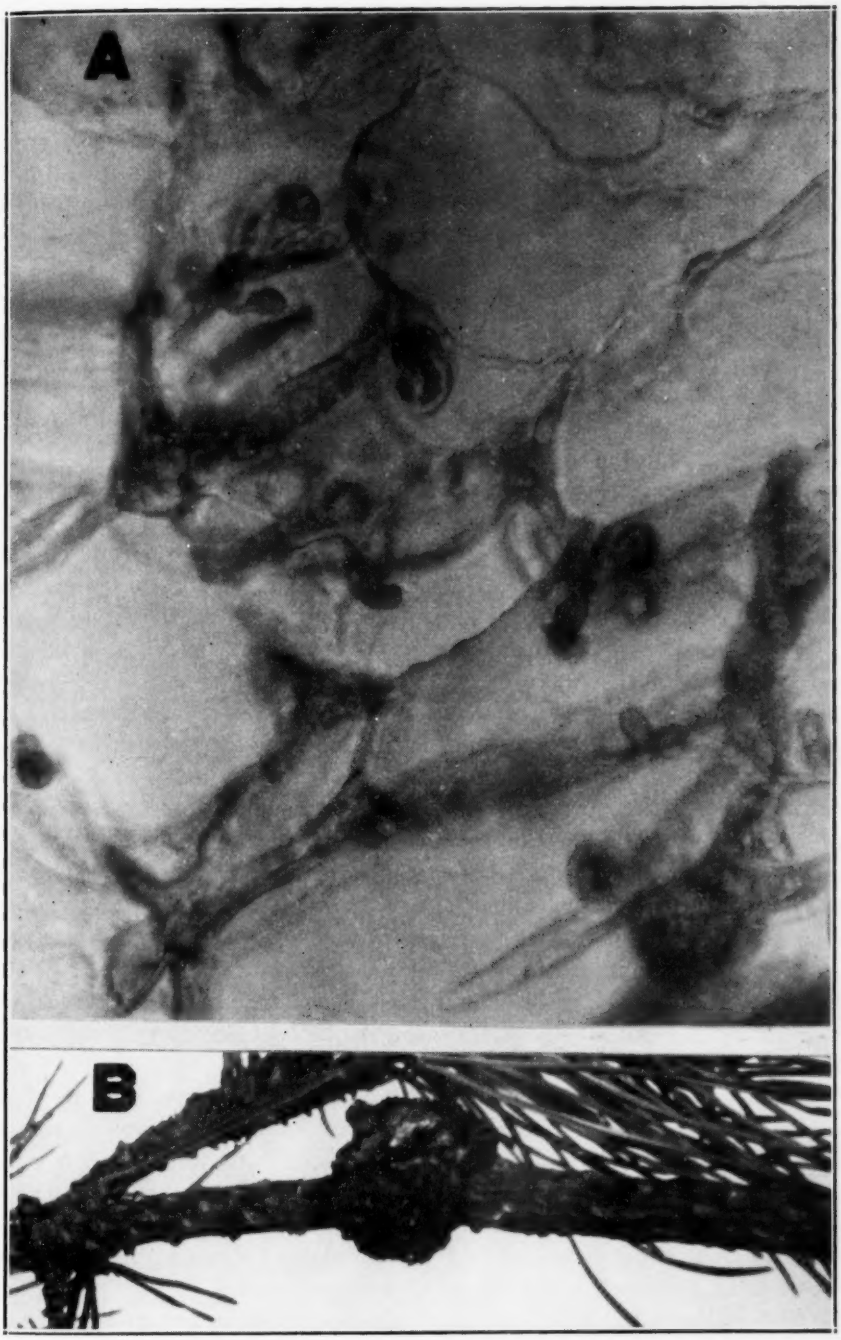

Fig. 2. A. Haustoria in tangential longitudinal section of bark of *Pinus sylvestris*. × 1500. B. Gall from which section shown in A was obtained. × 1.

observed. Young haustoria are usually straight, constricted at the point of passage through the cell wall, and distended inside the cell. In the ray cells the haustoria reach their most complete development.

The host cell and its nucleus showed no evidence that the parasite interfered with the normal growth of the cell. Stimulation toward gall formation was observed, but no relation between haustoria and increased cell division in bark cells was noted. Frequently, haustoria were no more numerous in areas where gall formation was stimulated than in other areas.

Microchemical tests on fresh sections made at the borderline of the advancing mycelium in infected plants showed an abundance of starch, fat, resin, and tannin in tissue recently infected and in adjoining uninfected tissue. In cells apparently infected for a considerable period a marked decrease in tannin was observed.

The examination of sections from inoculated pines in which infection had not resulted in the formation of galls, including all three species of pine used in these experiments, showed two significant host reactions: (1) defoliation, which resulted in no permanent injury to the host, and (2) death of leaders or other new growth. In sections from pines in which defoliation occurred without apparent permanent injury to the trees, rust mycelium was present in dead cortical cells that had been cut off from adjoining tissues by a cork layer. Infection apparently made no further progress in such trees.

In sections from other trees where portions of new growth were ultimately completely killed, mycelium penetrated through the cork tissue into other living tissue where it remained alive for a limited period of time. Many haustoria were produced by the old persistent hyphae. Other cork layers were formed, however, and cut off the infected tissue. Sometimes this process continued for a while, but as the mycelium advanced, the limits of the newly infected area were more narrowed than those of the previously infected area, and finally a cork layer completely surrounded and checked the growth of the mycelium. In no case did sections of trees with dead leaders or sections of temporarily defoliated trees show that the rust had succeeded in attaining the mutualistic relationship in the host necessary for the true establishment of rust mycelium in plant tissue.

In general, the details of the histology of the sections from trees in which rust mycelium failed to become permanently established were similar to those known to occur in tissues of trees recovering from wounds. From the point where the advancement of the rust mycelium appeared to be checked in the host tissue, the histological details in the present study were somewhat similar to those described by Hutchinson for his inoculations of *Pinus sylvestris* with repeating aeciospores of woodgate rust. He found that considerable pathological tissue is formed during the time the rust mycelium is present in the host, but concluded that transient infection indicates resistance of the host. In the trees which he called resistant, however, he reported no such pronounced reactions following infection as occurred in cer-

tain cases in the present experiments. Perhaps in the latter, where infection apparently had been arrested, the mycelium was not permanently checked and would advance further at some later time. It would then appear that extremely heavy infection was responsible for the reactions of the host resulting in defoliation or death of new growth and also for the delay in the establishment of a balanced relationship between the parasite and the host.

PHYSIOLOGICAL STUDIES

Limited observations were made on the inoculated pines concerning physiological phenomena associated with rust infection. Trees in which the leaders were killed and temporarily defoliated trees, obviously lost considerable chlorophyll-bearing surface and suffered from the limitation of photosynthesis, respiration, and transpiration. Certain chemical differences in cell contents may have had a bearing on physiological reactions. Microchemical tests mentioned under histological studies indicated that there were some chemical differences between infected and uninfected plants. Whether a rapid reaction of the fungus following infection in leaders or other new growth was responsible for the defoliation or death of trees was difficult, if not impossible, to determine. Some unbalanced relationship, however, must have existed after infection in order for temporary defoliation or death to have resulted. In trees in which rust mycelium had spread considerably, physiological disturbances apparently were not serious enough to cause such conspicuous host reactions, but witches' brooms have been formed in some cases. No satisfactory explanation can be given here for the physiological reactions associated with infection.

DISCUSSION

There are on record only three or four species of the pinicolous, caulicolous, gall-forming genus *Cronartium*, the aeciospores of which will reinfect pines and which therefore are microcyclic, obligately or facultatively as the case may be. One of these is the European *Peridermium pini* on *Pinus sylvestris*, for which no alternate host is known. The second is the *C. harknessii-C. coleosporioides* complex on several hard pines (6, 26, 27, 28). Then there is the case of the species on *Pinus radiata* and *P. attenuata* in the western United States (26, 27), doubtfully referred to *Cronartium quercuum* (6, pp. 24, 26, 30). And finally there is the woodgate rust discovered by York on *Pinus sylvestris* in New York State, for which again no alternate host is known, and which was referred by Arthur to *Cronartium quercuum* (6, p. 26) after this investigation was started.

The present studies were undertaken primarily to determine whether the aeciospores collected from pines were capable of reinfecting the pine hosts. Aeciospores from galls on *Pinus banksiana* and *P. rigida* growing within a few yards of oaks bearing uredia and telia were inoculated onto *Pinus sylvestris*, *P. banksiana* and *P. rigida*. Studies of the spores used in the inoculations leave no doubt that they are true binucleate aeciospores which germinate by means of germ tubes.

If the aeciospores used in these investigations were produced by *Cronartium quercuum*, and this species on the hard pines in the eastern United States has not previously been known to produce repeating aeciospores, the following questions arise:

1. Is the Peridermium stage of *C. quercuum* facultatively autoecious in general? That is, are the aeciospores capable of reinfecting pines instead of, or possibly as well as, oaks, the usual uredial hosts of this rust? 2. Have we here a strain of *C. quercuum* on a certain pine host, which has become confirmedly microcyclic?—that is, one that can infect pines only and oaks not at all? 3. Or have we a purely artificial situation created by the conditions of inoculation experiments? It is regrettable that these questions cannot at present be answered. It can be stated only that it has been found that aeciospores of this rust from two pine hosts in two localities, in both of which infected oak leaves were found, can reinfect the usual aecial host under experimental conditions.

As to the bearing of these results upon the problem of the identity of the woodgate rust, likewise no conclusions can at present be drawn, because the infections and galls produced did not mature sufficiently to enable one to compare them with typical woodgate rust galls. It can be said only that there still remains a possibility that woodgate rust may be microcyclic strain of *C. quercuum*, as Arthur concludes on morphological grounds.

While it must be admitted that few data are available with respect to the prevalence of microcyclic forest-tree rusts, and especially as to facultative microcyclicism in rusts that are normally macrocyclic, and while the data presented in this paper are meagre, on the other hand it is interesting to speculate upon the bearing of these facts upon the evolution of rusts in general. In view of the "general tendency towards shortening of the life cycle . . . in cooler climates and higher latitudes" (5, p. 185), it would be interesting to make observations and perform experiments upon various rusts in their more northerly habitats. *Cronartium quercuum*, for example, should be investigated in Canada. Furthermore, this species should be of special interest because of the possibility of its plasticity with regard to the evolution of microcyclic races or species. Meinecke's successful inoculations with aeciospores of a species on west coast and mountain pines, doubtfully referred to *C. quercuum*, and the successful inoculations reported herein with aeciospores of what may be this species, are cases in point.

Repetition of experiments presented here and further investigations along these lines are desirable from a variety of points of view. Such studies are expensive, time consuming, and difficult to evaluate, but these facts should not be permitted to prevent much needed further critical investigation of the heteroecious rusts of the genus *Cronartium*. At the present stage of our knowledge of the subject, one is entitled to wonder what may be the situation in the other heteroecious forest-tree rusts not investigated, even though failure has marked all attempts to show the repeating character of the aeciospores of *C. ribicola*. Furthermore, it should be kept in mind that

there is a very important practical aspect of the subject. Facultative autoecism of aeciospores in an economically important rust would present a problem of grave importance in the practice of forest pathology, since control of the parasite would be materially handicapped by its ability to spread after the elimination of the alternate host.

SUMMARY

Inoculations of *Pinus sylvestris*, *P. banksiana*, and *P. rigida* were made with aeciospores of a gall-forming Peridermium, presumably referable to *Cronartium quercuum* (see footnote 4), from *P. banksiana* and *P. rigida*. The aeciospores used in the inoculation experiments were binucleate. The mycelia giving rise to the aecia that bore the spores were uninucleate. Germination of aeciospores always showed them to form germ tubes. No basidia or secondary spores were observed.

The inoculations resulted in the infection of all hosts. Only in the case of *Pinus sylvestris* inoculated with aeciospores from *P. banksiana*, however, were galls formed. No aecia or pycnia appeared on the galls. On all species of pine inoculated with aeciospores from all collections, numerous cases of slight roughening, swelling, or discoloring of the bark, which did not result in the formation of galls, were observed. Histological studies of discolored bark showed the presence of typical rust mycelia and haustoria.

Microchemical tests made of the trees showing infection have indicated certain differences between the various plants after infection. No significant differences were apparent prior to infection. In some infected plants bark discoloration, abundant mycelia, haustoria, galls, and witches' brooms were observed. In other infected plants defoliation and death of leaders or complete necrosis have resulted.

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THE SPREAD OF VIRUS DISEASES OF THE YELLOWS TYPE UNDER FIELD CONDITIONS

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INTRODUCTION

The various factors related to wind and weather, which vary in an indeterminable manner and which influence insect behavior, probably are considered as insurmountable barriers to a rational consideration of the spread of insect-transmitted diseases and the dissemination of the viriferous[1] agents. However that may be, an understanding of the pattern produced under field conditions would be of obvious value to plant pathologists, and it seems likely that several applications may be made of an adequate description of the disease spread and virus dissemination. In this paper the writers are attempting such a rational consideration of disease spread in a special case of the insect transmission of viruses of the yellows type. Although an evaluation of constants cannot be undertaken, the general equation developed seems to describe adequately the distribution pattern, inasmuch as data we have on hand satisfy the demands of the equation. Unfortunately, observations reported have been restricted to the dissemination of yellow dwarf of potatoes into potato fields,[2] and of the dissemination of aster yellows into endive and lettuce beds,[3] but the results seem interesting enough to publish.

THEORETICAL

In a consideration of the spread of a virus disease by leaf hoppers, there are two extreme cases that may lend themselves to some sort of analysis; the one where the spread from plant to plant within the field is important, and the second where the spread of the disease from plant to plant within the field in question is of no importance and where the bulk of the virus effective in the spread has come from sources outside the field. This situation would be realized if the virus were picked up by the insect with difficulty in the field in question (as, for example, yellow dwarf in a potato field) or if the great majority of invading insects were initially viriferous. By spread from plant to plant within the field we have in mind that increased spread of the disease attributable to the increased percentage of viriferous insects resulting directly from insect feeding on plants that were infected

[1] So far as we have been able to learn, the term *viriferous* was used initially by C. F. Stahl and Eubanks Carsner (Jour. Econ. Entomology **16**: 476–479. 1923) to connote a virus-bearing agent. They later adopted the term *viruliferous* for the same purpose (Jour. Agr. Res. **28**: 297–319. 1924) because it had been used in the medical literature and they wished to avoid the duplication of terms. We like the term *viriferous* and are reintroducing it at the risk of being criticized for nonconformity.

[2] Hansing, E. D. A study of the control of the yellow dwarf disease of potatoes. Ph.D. thesis, Cornell University. Unpublished.

[3] Linn, M. B. The yellows disease of lettuce and endive. New York (Cornell) Agr. Exp. Stat. Bull. 742, 1940.

as a consequence of the invasion. A limitation in these deductions is that *only* the case where the spread of the disease from plant to plant within the field is of no importance shall be considered. One may feel justified in considering that the bulk of the effective inocula was brought into the field in the two cases concerned because, on the one hand, observations show that it is infrequent that leaf hoppers become viriferous from feeding on a yellow dwarf potato plant, and on the other hand, there is good reason to believe that the percentage of viriferous aster-yellows leaf hoppers that invaded our various fields was very high.

In any analysis of a mathematical nature some assumptions are necessary in setting up equations. Should the hypothesized phenomena agree well with observation, one may conclude that the assumptions made were reasonable, and that he has given expression to a natural law, which, however, should be accepted only after extensive study. Two basic assumptions are made in the consideration of virus dissemination under field conditions, one of which is that the movements of the leaf hoppers involved is random. The second assumption, which may not stand too close scrutiny, is that the number of plants in a given area that become infected is proportional to the number of insects in that area, to the average number of different plants an insect will feed on per unit of time, and to the time. That is, the number of plants that become infected is proportional to the number that have been fed upon. The difficulty with this assumption is that there is in any case only a limited number of plants per unit area, and that, as time goes on, the number of hitherto unmolested plants becomes less and less, and the probability of an infection per unit time consequently becomes less and less. This factor is disregarded in the following deductions because its consideration gives rise to mathematical difficulties that are indeed formidable, but chiefly because it does not seem to have too great a bearing on the problem, as shall be seen later.

In order to facilitate the mathematical treatment one may direct his attention to an isolated unit area of the field, having its edges parallel to the edges of the field, and consider the events that occur in that area, and then sum up the events for the entire field and for the total duration of the experiment. In the invasion of a field where the movements of the individual insects is random, there will be more insects entering the unit area on the side near the source of insects per unit of time than are leaving it from the opposite side, and there will be, consequently, a net gain in insect population per unit time. The component of the movements of the insects parallel to the edge of the field near the reservoir cancel out, so that one need only consider the flow of insects in the direction perpendicular to the reservoir edge of the field. Mathematically expressed,

$$-\frac{\partial c}{\partial x}K$$

insects enter the unit area per unit time where c is the number of insects per unit area, x is the coordinate in the direction of insect flow, and K is

average distance an insect will move in the x direction in our unit time interval, and $-\frac{\partial c}{\partial x}$ is the density gradient. The number of insects leaving the unit area per unit time then is

$$-K\left[\frac{\partial c}{\partial x}-\left(-\frac{\partial}{\partial x}\left(\frac{\partial c}{\partial x}\right)\right)\right]$$

and the difference between these two expressions represents the net gain in insect population per unit time. The time rate of change of insect population density in the unit area may be expressed as

$$\frac{\partial c}{\partial t}=K\frac{\partial^2 c}{\partial x^2} \tag{1}$$

The concept involved in the development of equation (1) is that the migration of insects in mass may be considered as comparable to the flow of a compressible fluid. However, a more rigorous derivation may be obtained from considerations involving some of the principles of the Boltzmann statistics in the case of a 2-dimensional gas, where the average length of insect hop corresponds to the mean free path of the gaseous molecules. Equation (1) is, in reality, the general equation for the diffusion process in one direction, and there is implied a very close analogy between the behavior of the individual insect and the thermal motion of an individual fluid molecule or colloid particle, and K corresponds to the diffusion constant. There may be the objection raised that the assumption involved in the derivation of equation (1) is not valid because of the effects of wind and various other factors on the migration of insects. The answer is that the effects of these various factors appears to be one of superposition, and the general pattern produced over a reasonable period of time is not affected. That is, the general pattern produced will be the same whether the insect migration be rapid or slow, and the equation will hold true for any element of time, providing the movements of the insects be random. The effect of the factors related to weather that affect the insect migration will have an effect on the value of K in equation (1), and the value of K is taken over a long period of time.

Equation (1) has to do with the behavior of the insects, whereas the primary interest in this paper is in the spread of the disease. Since the disease is spread by the insect, there is a very close relationship between the two. The connection between the variation of the insect population and the variation of amount of disease occurring in the unit area may be shown in the following manner. If, for the moment, one may regard the unit area as containing a constant insect population, one may write, in keeping with the second basic assumption,

$$I=entR \tag{2}$$

where I = number of plants that have become infected in time t
c = number of insects per unit area
n = average number of different plants an insect will feed on per unit of time
t = time
R = proportionality constant.

Now

$$\frac{\partial I}{\partial t} = enR + \frac{\partial e}{\partial t} tnR \tag{3}$$

and

$$\frac{\partial^2 I}{\partial x^2} = \frac{\partial^2 e}{\partial x^2} ntR \tag{4}$$

and one may substitute equations (3) and (4) in equation (1) to obtain

$$\frac{\partial I}{\partial t} - \frac{I}{t} = K\frac{\partial^2 I}{\partial x^2}. \tag{5}$$

Equation (5) is the differential equation for the spread of a disease under field conditions, subject to the limitations imposed.

The form of the solution of equation (5) that is desired will depend on the experimental set up; in the field of physics the experimental conditions are spoken of as the boundary conditions. In the present considerations we shall take as the boundary conditions: (1) the insect population in the freshly plowed field at the start of the experiment is negligible, (2) the area that serves as a reservoir of insects has a very large supply of insects and that the invasion of the field in question does not appreciably lessen the population density in the reservoir, (3) attention will be confined to regions not too near the edges of the field. Under these boundary conditions a solution of equation (5) that appears satisfactory is

$$I = Kte^{st - \sqrt{s}\,x} \tag{6}$$

where s and $\sqrt{s}$ are constants of integration and where e is the Napierian constant.

One may, if he desire, divide both sides of equation (6) by the number of plants in the unit area without affecting the validity of the equation, and thus handle the data in terms of fractions of, or percentages of, plants infected.

Equation (6) may be written in the logarithmic form

$$\log I = \log K + \log t + st - \sqrt{s}\,x \tag{7}$$

and it is seen that two demands made by equation (7) are that, at any given time log I be proportional to the distance into the field, and that, at any point in the field, log I be proportional to log t + t. In the case where the variation of percentage infection is observed for a definite time, $-\sqrt{s}$ is the slope of the curve, and, at the intercept where x = 0, log I = log K + log t + st. In the case where we fix our attention to a given area in the field and observe the variation of percentage infection with time, and plot log I – log t against t, the slope of the curve is s. The value of K may be determined by observing the behavior of the insects in question under field conditions.

EXPERIMENTAL

The several experiments that have been conducted under field conditions permitting a test of these two demands by equation (7) may be described briefly. The first field plot in studies with yellow dwarf of potatoes in up-state New York is shown diagrammatically in figure 1, with

the rows planted along contour lines. Between the well-worn lane, at the top of the figure, and the potato plot there was a 10-foot uncultivated strip in which there occurred weeds, clover, and some clover leaf hoppers. Data were obtained by permitting the plants to mature and then tuber indexing representative samples in the greenhouse. The old meadow was densely populated with viriferous clover leaf hoppers. The distance into the field was measured at right angles to the contour lines, and the row width was taken as the unit of length (about 18″). The time was necessarily the full season. In this case, as well as the various others, yellow-dwarf-free seed stock was used.

There was nothing unusual about the other potato plots except that they were adjacent to fields in which virus-bearing clover leaf hoppers[4] occurred.

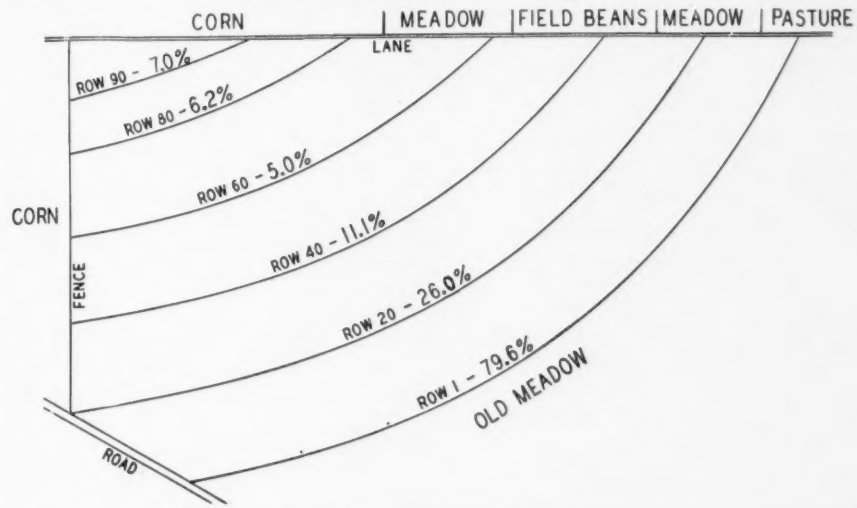

FIG. 1. The field plot in the first experiment on the dissemination of the yellow dwarf virus into potato fields.

The work with aster yellows was carried out on Staten Island, N. Y. A typical field plot is the endive plot shown in figure 2. The weeds to the left served as a reservoir of viriferous aster-yellows leaf hoppers; note that the fraction of plants diseased decreases as the distance into the field increases.

In figure 3 we have plotted log I, where I is the percentage of plants infected with yellow dwarf, against the distance into the various potato fields involved in the experiments. The top curve in figure 1 was the one obtained from the data taken in our first field plot, namely, the field diagrammatically represented in figure 1. The influence of the insects migrating into the field from the lane may be readily seen; there was a decrease in infection from row 90 to row number 60. It is evident from figure 3 that, with the exception of one field, the invasion by the insects was from both edges of each field. Note that in each instance one of the demands of equation (7) is met; log I is proportional to the distance into the field.

[4] For details see the article by Hansing, footnote 2.

The situation is the same in the case where aster yellows spread into endive beds from the adjacent weed patches. Log I, where I is the percentage of plants infected, is plotted, in figure 4, against the distance into

FIG. 2. Appearance of the typical endive bed in the case where the aster-yellows virus has spread from the adjacent weed patch.

the field for four separate experiments. The demand of equation (7) that log I be proportional to the distance into the field is met by these data.

In any experiment where the insect population is held constant, $I = cntR$,

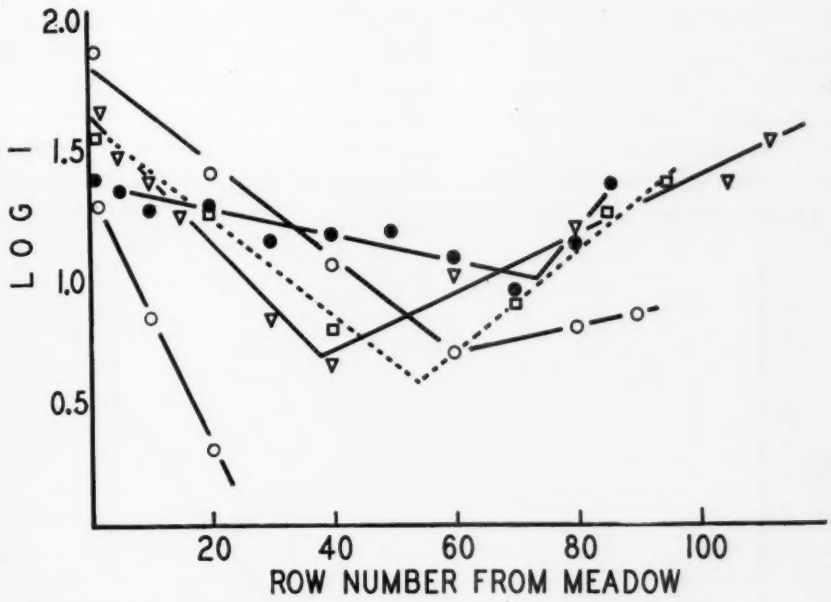

FIG. 3. The variation of the percentage of plants infected, with distance into the field, in the case of yellow-dwarf virus spreading into potato fields.

if the second basic assumption be valid, and the amount of infection occurring in the area under observation should vary directly with the time, provided the time interval is short enough so that the influence of a new brood is not of importance. The validity of the assumption involved in equation (2) was tested in a set of experiments where areas in lettuce fields containing

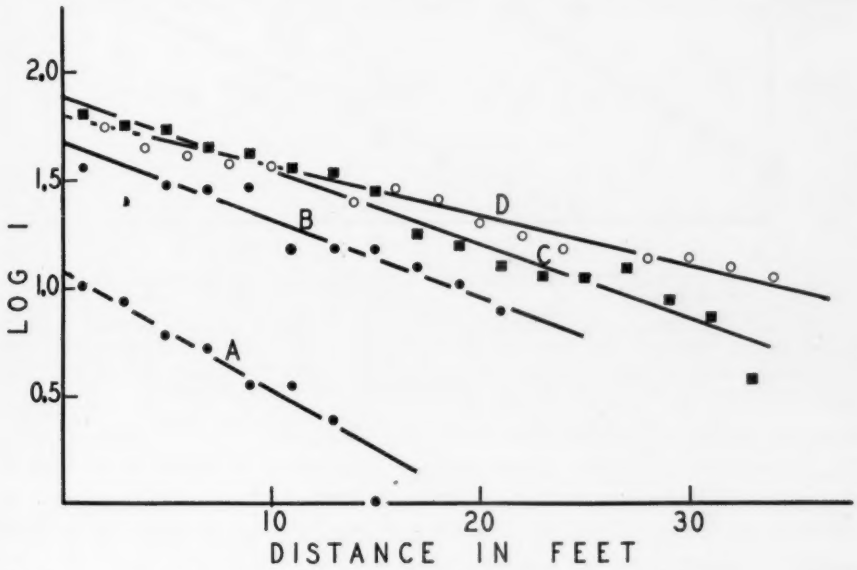

FIG. 4. The variation of the percentage of plants infected with distance into the field in the case of aster-yellows virus spreading into endive beds.

aster-yellows leaf hoppers were enclosed in a manner that precluded any substantial insect invasion. The data for the time increase in infection in the enclosure are plotted against the time in curve A of figure 5, and we see the justification for our second basic assumption.

In keeping with this line of thought, a further set of two experiments was conducted where observations were made on the time rate of infection in a fixed area to which insects had free access. The data, averaged for the

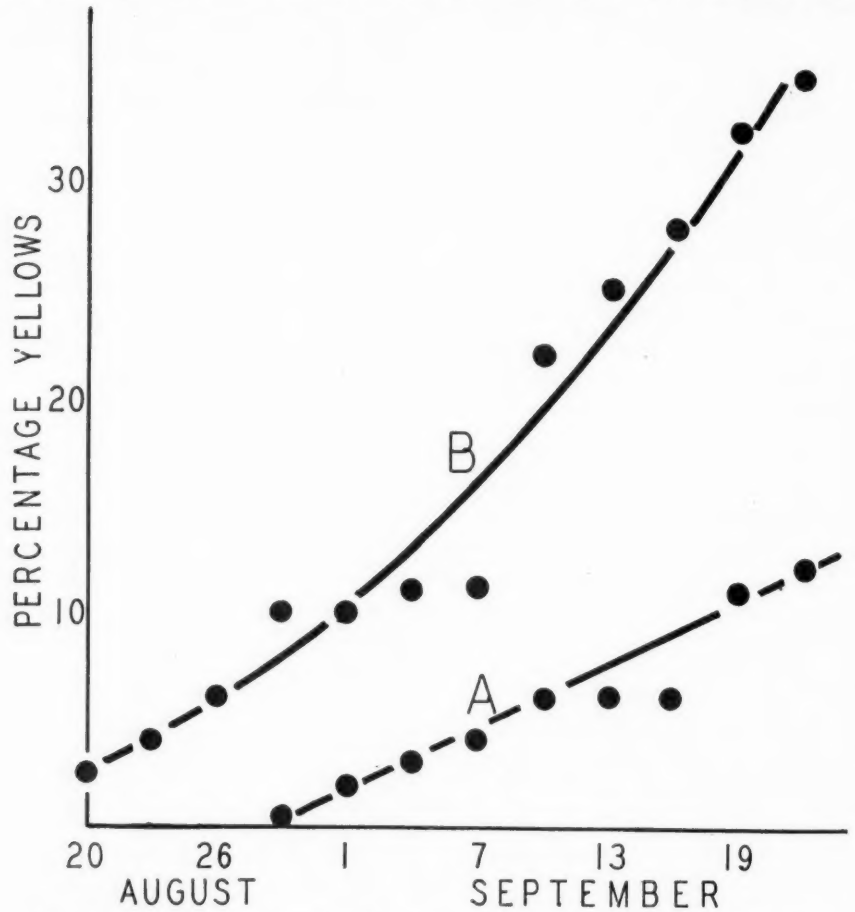

FIG. 5. The time variation of the percentage of plants infected in fixed areas in lettuce beds in the case of the spread of aster-yellows virus. Cure A is for the case where the invasion of the area by the leaf hoppers was restricted; curve B is for the case where there was no restriction imposed on the invasion of the area by the insects.

two experiments, are plotted in curve B of figure 5. The amount of infection occurring in this case is not a linear function of time, but rather varies somewhat in the manner demanded by equation (6). A concurrence in the demand of equation (7) is shown in figure 6 where log I is plotted against $t + \log t$, with the resulting straight line.

In brief, then, a differential equation describing the dissemination of

viruses of the yellows type under field conditions has been derived, it has been integrated, and data obtained in a few experiments satisfy two demands of the integrated form of the equation. As has been pointed out, at the present time one is not in position to give values for the constants in the equation, and indeed each different experiment will yield different constants. In reality the full usefulness of this treatment may come when

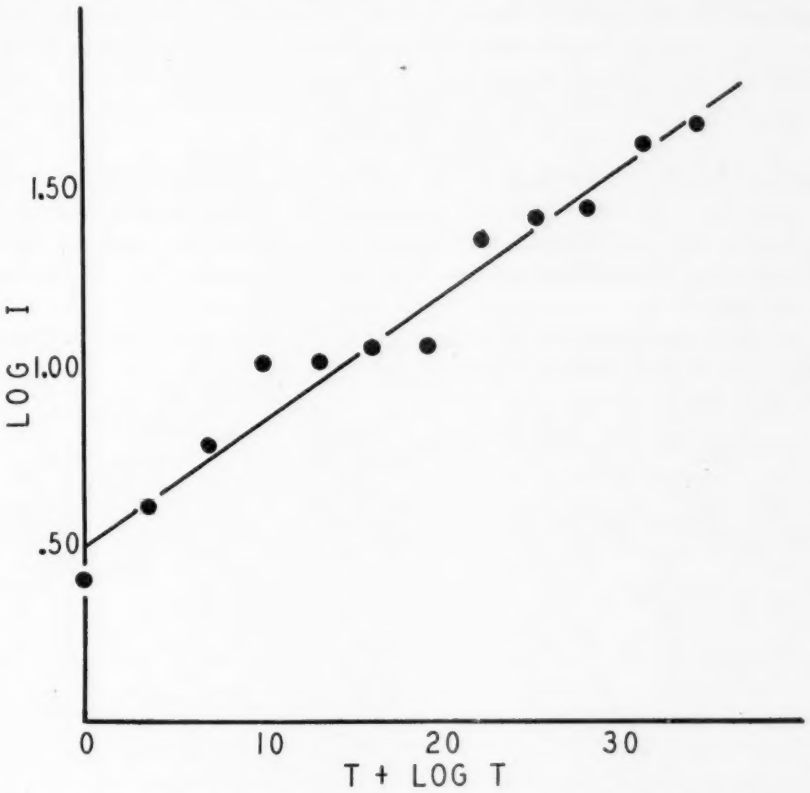

FIG. 6. The log of the percentage infection is plotted against the time + log time in a fixed area of a lettuce bed in the case where the invasion of the bed by the leaf hoppers was not restricted.

sufficient experimental work has been conducted so that one may obtain the most probable value for each of the three constants in the case of each species of insect and each crop, in which event much of the guess work in connection with the placement of field plots relative to possible insect reservoirs as a means of control of the disease will have been removed.

SUMMARY

A differential equation

$$\frac{\partial I}{\partial t} - \frac{I}{t} = K \frac{\partial^2 I}{\partial x^2}$$

where $I = \%$ infection
$t =$ time
$x =$ distance into field
$K =$ constant

has been developed that describes the spread of virus diseases of the yellows type under field conditions. The assumptions involved in the derivation of this equation were that the movements of the insects involved is random, that the number of plants that become infected is proportional to the number of plants that have been fed upon, and that spread from plant to plant within the field is of small importance.

The integrated form of the equation is

$$I = Kt^{st - \sqrt{s}\,x}$$

where s and $\sqrt{s}$ are constants of integration. The boundary conditions that were set up were that the insect population in the newly plowed field was negligible, that the insect reservoir was not substantially depleted during the course of the experiment, and that the effects at the ends of the fields be neglected.

Several data obtained under field conditions satisfy two of the demands made by the integrated form of the equation.

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CLAVICEPS YANAGAWAENSIS IN IMPORTED SEED OF JAPANESE LAWN GRASS

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In 1939 the late Dr. A. J. Pieters received a sample of seed of Japanese lawn grass, *Zoysia japonica* Steud., from Japan that contained a high percentage of sclerotia of *Claviceps yanagawaensis* Togashi (Fig. 1). The sample was tested for viability in the seed laboratory. In the first germination test, the nontreated seed was placed in moist blotters and incubated at alternating temperatures of 20–35° C. Only 36 per cent of the seed germinated. Two other samples were treated with a 75 per cent solution of sulphuric acid, the one (Lot 1) for 20 minutes and the other (Lot 2) for 30 minutes. These treatments both improved the germination very significantly. Lot 1 germinated 58.0 per cent and lot 2 germinated 58.5 per cent.[1]

FIG. 1. A. Portion of seed sample of *Zoysia japonica* containing ergot sclerotia. B. Sclerotia of *Claviceps yanagawaensis*. ×1½.

It was observed that the sulphuric acid treatment had so charred the ergot sclerotia as to suggest their having been killed. To determine this, 3 sets of 25 sclerotia from each of lot 1 and lot 2, treated, and from lot 3, nontreated, were placed on the surface of sterilized soil in 2-in. pots. The three 2-in. pots of each set, containing sclerotia from lots 1, 2, and 3, respectively, were sunk in soil in separate 6-in. pots. The three 6-in. pots then were sunk to their tops in soil out of doors, covered with inverted clay saucers, and banked with soil to prevent excessive drying. With such an arrangement there were 25 sclerotia from each lot in each 6-inch pot under

[1] The writer is indebted to Albina F. Musil for making these germination tests.

identical conditions, and there were three replications, making 75 sclerotia from each lot included in this trial.

The sclerotia were placed as described above and the pots sunk out of

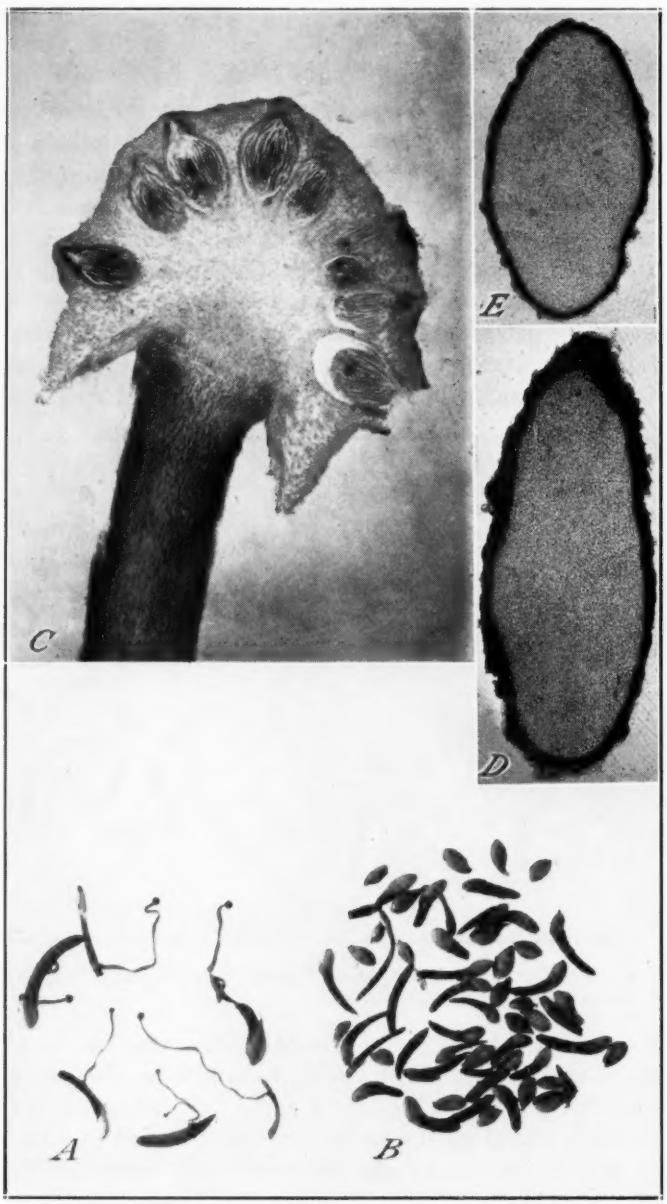

FIG. 2. Sclerotia and stromatic head of *Claviceps yanagawaensis:* A. Germinating sclerotia. ×2. B. Portion of seed sample treated 20 minutes in a 75 per cent solution of sulphuric acid showing charred sclerotia. ×2. C. Section through head and upper portion of stipe showing perithecia embedded in the head. ×75. D. and E. Cross sections of sclerotia, showing flattening. ×40.

doors on November 30, 1939, and were left undisturbed until the following spring. Counts of germinated sclerotia were made May 17, 1940. None of the treated sclerotia had germinated, while 9, 14, and 13 sclerotia, respectively, had germinated (Fig. 2, A) in the 3 nontreated lots. In other words, almost 50 per cent of the nontreated ones germinated, while none of the treated sclerotia were found to be viable.

The following year, using the same source of ergot, an attempt was made to determine the minimum treatment necessary to prevent germination of the sclerotia. The sclerotia were treated from 1 up to 30 minutes with concentrated sulphuric acid and were placed out of doors, as before, along with nontreated sclerotia. Neither the treated nor nontreated sclerotia germinated the following spring however, which indicated that they were comparatively short-lived.

THE FUNGUS

The sclerotia were 0.25 to 1.5 mm. long and 0.5 to 1 mm. wide, flattened (Fig. 2, D and E), usually slightly curved, and, for the most part, grayish-violet. Some of the sclerotia had a yellowish-green gloss over the surface that made them appear lighter in color. It was rather striking to find the shiny flattened, indurated glumes of the host attached to almost all of the sclerotia (Fig. 1).

The sclerotia germinated by producing usually 1 stroma, but occasionally 2, from the apical third of the sclerotium (Fig. 2, A). Under different conditions, the number of stromata may be greater, as shown by Togashi.[2] The stipes measured 4 to 16 mm. in length and 0.3 to 0.5 mm. in width. The length for the stipes exceeded, while their width was somewhat less than the measurements given by Togashi. This difference may be due to the fact that the writer germinated the sclerotia in the dark. Similarly, when the writer germinated sclerotia of *Claviceps paspali* S. and H. in the dark, the stipes were much longer than those from sclerotia germinated in the light.

Measurements of the heads (Fig. 2, C), perithecia, asci, and ascospores fell well within the ranges given by Togashi, which were, 0.3–1.0 mm. × 0.4–1.5 mm.; 180–320 × 70–190 μ; 85–165 × 4.0–8.0 μ; and 75–135 × 1.0–2.25 μ, respectively. From the shape, size, and color of the sclerotia, and measurements of the other reproductive bodies, the ergot found in the *Zoysia japonica* seed sample from Japan agrees well with Togashi's description of *Claviceps yanagawaensis*.

Heads of *Claviceps yanagawaensis*, containing mature spores, were crushed in sterilized distilled water. This material was then used to inoculate heads of a rye hybrid that was known to be highly susceptible to *C. purpurea* (Fr.) Tul. The rye heads were dipped in the inoculum and rolled between the fingers to insure contact of the spores with the pistil. Of the thirty heads inoculated, 10 were placed in a moist chamber for 48 hours, 10 were covered with glassine bags, while the remaining 10 were left uncovered. No infection resulted.

[2] Togashi, Kogo. New species of parasitic fungi. I. Transactions of the Sapporo Natural History Society **14**: 280–285. 1936.

SUMMARY

Sclerotia of *Claviceps yanagawaensis* Tog. were found in seed of Japanese lawn grass imported from Japan. This fungus had not previously been reported in the United States.

Treating Japanese lawn-grass seed, containing ergot sclerotia, with a 75 per cent solution of sulphuric acid from 20 to 30 minutes killed the ergot sclerotia, whereas the germination of the seed was improved. It appears from the one test made that if such contaminated seed is treated with sulphuric acid it may be distributed for planting without the danger of introducing a new grass disease into the United States.

A rye hybrid, known to be highly susceptible to *Claviceps purpurea*, failed to become infected when inoculated with mature ascospores of *C. yanagawaensis*.

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RESISTANCE OF CERTAIN POTATO VARIETIES AND SEEDLING PROGENIES TO RING ROT

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(Accepted for publication February 6, 1942)

INTRODUCTION

Ring rot, caused by *Phytomonas sepedonica* (Spieckermann) Magrou, is one of the major potato diseases of the United States and Canada. It was reported from Canada by Baribeau[5] in 1931 as prevalent in potato fields of the Province of Quebec. Ring rot was discovered in the United States in 1932 in a field of potatoes in Aroostook County, Maine[6] in which State it has spread rapidly. It has been reported present in 37 States, and the resultant losses in some instances have been heavy.[7]

The planting of disease-free seed potatoes and the use of sanitary methods to avoid contamination are now generally recommended for the control of ring rot. The results of attempts to follow these recommendations have been discouraging to many growers. It has been difficult to secure seed stocks that are entirely free from ring rot. A trace of disease in the seed often has been responsible for very rapid spread within a stock. Even when seemingly adequate precautions have been taken to prevent contamination, it has been difficult to control such spread within stocks of the commercial varieties in Aroostook County. Slightly infected seed potatoes are a real menace wherever susceptible varieties are grown.

The ring-rot problem could be solved, to some extent at least, by finding or breeding resistant or immune varieties. Work on this phase of the problem is under way, and some of the results thus far obtained are reported in this paper.

MATERIALS AND METHODS

As a preliminary study in breeding for resistance to ring rot, as many as possible of the existing potato varieties were tested. Fifty-four named domestic and foreign varieties and many unnamed seedling varieties, both American and foreign, were included in the test. In addition, a limited

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[5] Baribeau, Bernard. Bacterial wilt of potatoes. Can. Pl. Dis. Surv. Rept. **11**: 49. 1931.

[6] Bonde, Reiner. A bacterial wilt and soft rot of the potato in Maine. Phytopath. **27**: 106–108. 1937.

[7] Leach, J. G. Report of the Committee to Coordinate Research on New and Unusual Potato Diseases. Amer. Pot. Jour. **17**: 81–88. 1940.

number of the progenies of 3 crosses were tested to determine the inheritance of resistance.

The use of pure cultures of the ring-rot organism for inoculating potato tubers has not given reliable results in studies in Maine. Likewise hypodermic inoculations have been unsatisfactory. Therefore, a rather crude and laborious but effective method of inoculation was used. Slices of infected Green Mountain tubers were rubbed on the freshly cut surfaces of healthy seed pieces. The inoculated seed pieces were planted immediately in the field. The plants were dug after frost had killed the vines, and the individual tubers were cut into slices that were observed carefully for symptoms of ring rot.

During the winter of 1939–40 inoculations of 3 to 5 seed pieces of each variety were made in the greenhouse. Approximately 10 seed pieces of each were tested again in 1940 under field conditions. The varieties that showed no infection or only a slight amount as a result of these inoculations were tested again in 1941. The experiment in 1941 included 4 plots of each variety using 5 inoculated seed pieces for each plot.

Control inoculations were made with the susceptible Green Mountain variety, one lot for every 5 variety plots. Symptoms of ring rot are generally quite pronounced in both vines and tubers of the Green Mountain.

RESULTS

Varietal Reactions to Ring Rot. The data obtained from testing different varieties for resistance to ring rot are summarized in table 1. It may be noted that most of the 54 named foreign and domestic varieties used in this experiment were ring-rot susceptible when inoculated by the described methods. Only two of them appeared to be highly resistant.

Friso, an apparently resistant one, has been inoculated 10 times in separate experiments, and to date no infected tubers have been found. Since all 20 separate control lots of similarly inoculated Green Mountain seed pieces produced the disease in high amounts, the indication is that the Friso variety is highly resistant. This variety was obtained from the Wageningen Experiment Station through the Netherlands Legation, and is said to be resistant to leaf roll also. A British variety, President, is apparently resistant. It has been tested for the past 3 years, and no symptoms of the disease have been observed. Four selections from the cross President × Katahdin, likewise, escaped infection, also one from the cross 41956 × Earlaine. Another, from Earlaine × 43055, showed only 4 per cent ring rot.

Parent and Progeny Reactions. The data for the reactions to ring rot of Green Mountain controls, of parents used in crosses, and 3 progenies are given in table 2.

Green Mountain was used as a control because of its extreme susceptibility. President, one of the few resistant varieties, has been used quite extensively as a female parent, but, so far, has not produced enough viable pollen to make it useful as a male parent. Katahdin, a susceptible variety,

TABLE 1.—*Reaction to ring rot of American, foreign, and seedling potato varieties. Tested in Maine for several years*

Variety	Seed pieces inoculated	Plants infected
	Number	*Per cent*
Ackersegen	30	77
American Giant	20	75
Albion	10	30
Arran Cairn	10	90
Arran Consul	40	50
Arran Scout	10	90
Arran Victory	20	40
Bally Doon	56	70
Bevelander	20	50
Burbank	20	75
Charles Downing	10	60
Chippewa	20	90
Columbia Russet	28	64
Donard	20	40
Earlaine	10	40
Earlaine No. 2	19	95
Early Blue	15	67
Early Ohio	35	49
Early Rose	10	90
Ekishirazu	3	100
Flava	10	40
Friso	50	0
Garnet Chile	2	100
Gold Adler	10	30
Golden	10	50
Golden Wonder	18	78
Green Mountain (control)	100	90
Harmony Beauty	20	75
Houma	20	75
Irish Cobbler	20	90
Katahdin	40	55
Kerr's Pink	20	30
Mesaba	28	68
Noordeling	10	70
Nordeland	10	50
Norkota	20	80
Parnassia	30	70
Pennigan	10	40
Perfect Peach Blow	18	61
Pontiac	19	63
President	48	0
Prolific (Brown Beauty)	10	60
Richter's Jubel	30	67
Russet Burbank	15	87
Russet Rural	20	85
Sebago	20	55
Sequoia	20	90
Shamrock	30	60
Spaulding Rose	25	80

TABLE 1.—(*Continued*)

Variety	Seed pieces inoculated	Plants infected
	Number	*Per cent*
Triumph (from Maine)	28	54
Triumph Nebraska Strain 1	20	30
Triumph Nebraska Strain 2	20	50
Triumph Nebraska Strain 3	20	30
Triumph Nebraska Strain 4	20	40
Triumph Nebraska Strain 5	20	100
Up-To-Date	31	58
Warba	25	80
White Gold	20	45
White Rose	10	70
Ten unnamed varieties secured from McGill and Smith, Ayr, Scotland	175	40–100[a]
Six unnamed varieties secured from U. S. S. R. Institute of Plant Breeding	105	50–80[a]
Five South American varieties from collections made by McMillan and Erlanson	102	50–90[a]
Eight blight-resistant selections from true seed sent by K. O. Müller, Berlin-Dahlem, Germany	80	10–60[a]
Twenty-three blight-resistant unnamed seedling varieties from Donald Reddick, Cornell University, Ithaca, N. Y.	470	30–80[a]
Six unnamed selected seedling varieties from the cross S. 41956 × Earlaine	110	0–60[a,b]
Two unnamed seedling varieties from cross Chippewa × Katahdin	20	40–70[a]
Four unnamed seedling varieties from cross President × Katahdin	44	0
One unnamed seedling variety from cross Earlaine × S. 43055	25	4

[a] Percentage range for the various seedlings in the group.
[b] One seedling contracted no disease in 17 inoculated plants.

has many characteristics that make it a desirable parent. It is highly self-fertile, is immune from virus A in the field, and produces relatively large yields of tubers with good market quality. Seedling variety 336–123 was selected from the cross President × Katahdin because of its resistance to late blight. It is susceptible to ring rot. Seedling variety 47156 was selected because of the good cooking quality and attractiveness of its tubers. It is susceptible to ring rot. Seedling variety 3895–13 was selected from a cross the seed of which came from K. O. Müller, Berlin-Dahlem, Germany. It is resistant to late blight, but, at present, its reaction to ring rot is not known. Earlaine, another susceptible variety, is early and produces viable pollen under a wide range of environmental conditions.

Nearly half of the seedling varieties from the resistant × susceptible cross President × Katahdin escaped infection. It is improbable that these were all chance escapes, since none of the Green Mountain lots were free

TABLE 2.—*Reactions to ring rot of Green Mountain checks, parents of crosses, and three progenies*

Variety or cross	Check and parent lots tested	Seedling[a] varieties	Percentage of lots in each class of infection[b]						Total percentage of lots showing infection
			0	1	2	3	4	5	
	Number	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Green Mountain	128		.....	.....	4.6	13.3	33.6	48.5	100
President	12		100.0	.....	.....	.....	.....	.....	0
Katahdin	20		.....	.....	20.0	40.0	20.0	20.0	100
336–123	20		.....	.....	10.0	15.0	25.0	50.0	100
47156	20		.....	.....	15.0	40.0	20.0	25.0	100
President × Katahdin		92	47.8	14.0	8.7	13.0	12.0	4.3	52
336–123 × 47156		20	5.0	5.0	15.0	40.0	20.0	15.0	95
3895–13 × Earlaine		22	4.0	9.0	36.0	32.0	14.0	5.0	96

[a] Test consisted of 4 lots of each seedling variety with 5 hills per lot.

[b] *Class of infection* *Infected plants grown from inoculated seed pieces*

0	=	0 per cent
1	=	1– 20 " "
2	=	21– 40 " "
3	=	41– 60 " "
4	=	61– 80 " "
5	=	81–100 " "

from the disease, and nearly half of the latter were found in the class showing 81 to 100 per cent of their tubers infected. It is evident that the President variety is heterozygous for resistance and that this character was transmitted to a relatively large percentage of the first generation progeny of the cross with the susceptible Katahdin. The data for the other two progenies, 336–123 × 47156 and 3895–13 × Earlaine, indicate that various degrees of resistance and susceptibility can be obtained from crosses between two susceptible varieties and that some of the segregates of the 336–123 × 47156 cross are more resistant than either parent.

DISCUSSION

Resistance to ring rot has been found in relatively few varieties, but it is interesting to note that it has been obtained from rather widely separated sources—Friso from the Netherlands, President from the British Empire, and a number of unrelated seedling varieties produced in the United States.

The resistant variety Friso has not yet been used as a parent because in both field and greenhouse tests its buds are abscissed and no viable pollen is produced. Attempts are being continued to find a set of environmental conditions under which it will produce flowers and pollen. President is apparently resistant, but neither Friso nor President is promising from the commercial standpoint. The most promising variety so far obtained is the selection from the cross Earlaine × 43055. This variety matures early, is resistant to virus A in the field, and produces a fair yield of tubers of excellent type in Maine. It is self-fertile and, if it continues to show the degree of resistance to ring rot indicated by the tests reported here, it should make an exceptional parent. It could be grown to advantage as a commercial crop in any section where earliness and resistance to ring rot are important factors in potato production.

Degrees of resistance and susceptibility were evident throughout the tests. For example, Green Mountain and Katahdin were both susceptible, but the Green Mountain tubers seemed to be more easily infected than those of the Katahdin. If the chi-square test for goodness of fit is applied to the data for these two varieties, using the numbers found in the various classes of infection instead of the percentages as given in table 2, there is not one chance in a hundred that these two varieties reacted alike. If resistant segregates can be obtained from crosses of 2 susceptible varieties and if nearly 50 per cent of the seedlings of a cross between a resistant and a susceptible variety are resistant, it should not be very difficult to produce varieties combining resistance with other characters of commercial importance.

SUMMARY

Ring rot, caused by *Phytomonas sepedonica* (Spieckermann) Magrou, is a major potato disease and in many instances is causing large losses to potato growers. Ring rot has spread rapidly and the presence of even a mere trace of the disease is a menace to the successful production of potatoes

of susceptible varieties. The availability of resistant or immune varieties would help greatly to control ring rot.

Fifty-four named foreign and American varieties and a number of unnamed seedling varieties were tested for resistance to ring rot. All but two of the named varieties were susceptible. These two were a variety from Holland named Friso and the British variety President.

An unnamed seedling variety from the progeny of a cross S. 41956 × Earlaine and another from a cross Earlaine × 43055 have been resistant. The latter has attractive tubers and other desirable commercial qualities.

Ninety-two selections from the progeny of a cross between the resistant variety President and the susceptible variety Katahdin were tested. Nearly half of these selections were resistant, and the others varied in degree of susceptibility. A few selections from a cross between 2 susceptible varieties were more resistant than either parent.

Judging from the limited data reported in the paper it should not be very difficult to produce varieties combining resistance to ring rot with other characters of commercial importance.

A STUDY OF SPREADERS FOR USE ON HOPS[1] IN THE FIELD CONTROL OF DOWNY MILDEW

G. R. HOERNER

(Accepted for publication January 22, 1942)

The germinating zoospores of the hop downy mildew fungus, (*Pseudoperonospora humuli* (Miy. and Tak.) Wils.) infect all aerial portions of hop plants by entering the host tissues, primarily through the stomata, which are most numerous on the under sides of the leaves.

Early-season infection of the leaves of the rapidly developing shoots often progresses upwards from the bases of the vines toward the tips, due, in part at least, to wind dissemination of the zoosporangia. Infected leaves

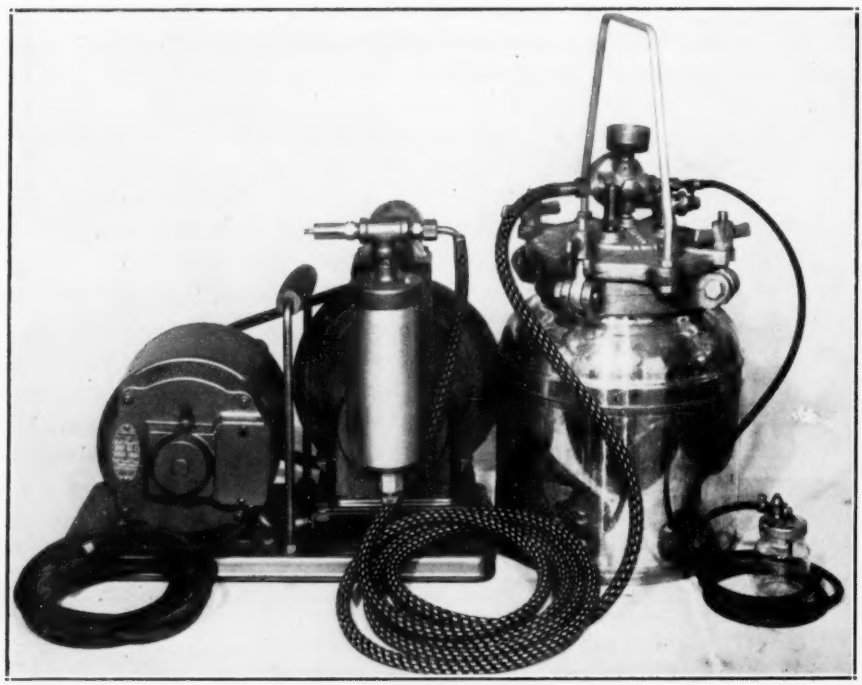

FIG. 1. Compressed-air laboratory sprayer.

occurring high up on the vines constitute a greater menace than do those nearer the ground. Because of their location they serve, throughout the growing season, as important sources of infection of other vital parts of the vines.

USE OF A SPREADER DESIRABLE

In applying liquid sprays for the control of the disease, it is highly de-

[1] Published as Technical Paper No. 395 with the approval of the Director, Oregon Agricultural Experiment Station.

sirable that a maximum area of the under sides of the leaves be covered by the protectant fungicide employed.

While some protection is doubtless afforded by fungicidal applications to the upper leaf surfaces, which are relatively easy to wet, particular attention should be directed toward securing a thorough and uniform coverage of the under sides of the leaves, which is not ordinarily accomplished unless a spreader is used.

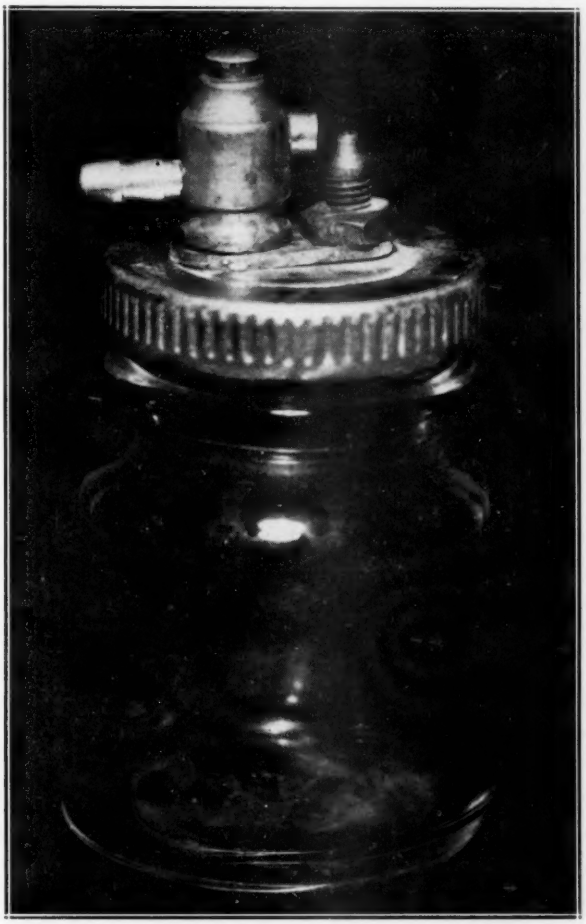

FIG. 2. Small sprayer used with air compressor.

The under sides of hop leaves are particularly difficult to wet, owing to the presence of numerous hairs and resin glands.

Numerous materials have been tested for their wetting and spreading capacity over a period of several years to determine what materials are most effective for the purpose and which are most readily available at minimum cost.

METHODS AND MATERIALS

The concentrations of all materials were reduced to the lowest points necessary to effect satisfactory coverage of the under sides of excised hop leaves in the laboratory. For this purpose a manually operated atomizer was first employed. Later in the course of the study a more satisfactory method of application was devised by employing the equipment shown (Fig. 1). It consists of a small air compressor, equipped with an oil and air filter, operated by a $\frac{1}{4}$-hp. electric motor. The air line from the compressor leads to a pressure tank equipped with a gauge that can be set at any desired

TABLE 1.—*Approximate amounts of spreader needed for 100 gallons of water*

Material	Lowest effective dilution	Material	Lowest effective dilution
Dry	*Amount (Pounds)*	*Liquid*	*Amount (Quarts, etc.)*
Glue, flakes	2	Raylig	6 quarts
Glutin flour-baking soda	2	Skim milk-lime	5 "
Neomerpin SS	2	New Lethane Spreader	2 "
Vatsol OS	2	S.-E.C Oil	2 "
Vatsol OTC	2	Tergitol Penetrant No. 08	1.5 "
Waste sulphite, powder	2	Areskap (Aresco)	1 "
XXX Spreader	2	Ortho (Del Monte) Spreader	1 "
Actin Wetting Compound	1	Spread-ol (Silmo No. 611)	1 "
Fluxit No. 1	1	Lethane Spreader (S51)	1 pint
Hydralene	1	Liquid-soap spreader	1 "
Kayso	1	Rosin-soap spreader	1 "
Skim-milk powder	1	Santomerse	12 ounces
Felbro Whale Oil Soap	0.5	Tergitol Penetrant No. 7	8 "
Fluxit No. 2	0.5	Grasselli Spreader (SS–3)	4 "
Lethalate	0.5	Tergitol Penetrant No. 4	4 "
Swift's Tar Soap, flakes	0.5		
Blood albumin-baking soda	0.25		
Gamboge	0.25		
Saponin	0.25		
Casein-lime	0.125		
Casein-ammonia	0.0625		

point up to 50 lb. per sq. in. The use of the tank avoids surging of the air flowing from the compressor to the sprayer. The small sprayer air line is attached to the pressure tank. The sprayer nozzle, regulating the pattern of the spray, is adjustable (Fig. 2). Various materials used can thus be compared under comparable conditions.

Spreaders which appeared promising were then applied to the leaves of hop plants growing in the greenhouse to observe possible injury, and those that seemed safe were finally applied, in certain instances, under field conditions by means of power sprayers of various mechanical designs at various pressures with a variety of nozzles. The age and condition of the vines, the equipment and pressures employed, as well as the fungicides to which the spreaders were added, all, affected in some degree, the final field formulae.

The data presented in this paper indicate the comparative effectiveness

of the materials listed below when applied alone to the under sides of hop leaves in laboratory or greenhouse only.

In the following table the amounts given under the heading "Lowest effective dilution" represent the approximate amounts of spreader needed for one hundred gallons of water.

The rosin-soap spreaders appeared particularly promising. At least 6 different formulae, which varied in the amount of rosin, caustic potash or soda, fish or corn oil, were compared. The final field formula adopted consists of a stock solution prepared from: 25 lb. rosin, 6 lb. caustic potash, and 25 gal. water. A minimum of 1 pint of stock solution is recommended with 100 gal. of any fungicide employed in the field.[2]

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[2] Hoerner, G. R. A Rosin-potash spreader for spraying hops for downy mildew control. Ore. Stat. Cir. Inf. 236, 2 pp. 1941.

METHOD OF OBTAINING PURE CULTURES OF CORTICIUM STEVENSII FROM SCLEROTIA

E. C. Tims and Frances Bonner

(Accepted for publication January 21, 1942)

Considerable difficulty often has been encountered in obtaining pure cultures of *Corticium stevensii* (Burt) from sclerotia at Louisiana State University. Other workers[1] also have had trouble obtaining pure cultures of the thread-blight organism by plating sclerotia from infected twigs. In connection with studies of fig leaf blights in Louisiana, attempts were made to develop a satisfactory method for obtaining pure cultures from thread-blight sclerotia.

Fig twigs of the current season's growth with numerous sclerotia of the thread-blight fungus on the surface were collected from several locations in southern Louisiana, and infected tung twigs were obtained from groves near Bogalusa, Louisiana. The sclerotia were removed from the twigs, sterilized or otherwise treated, and plated on water agar. Several treatment methods were used, including (1) rinsing in sterile water, (2) dipping in 95 per cent ethyl alcohol and flaming, (3) immersing in 1–1000 $HgCl_2$ solution in 50 per cent alcohol and rinsing in sterile water, and (4) immersing in the $HgCl_2$-alcohol solution, then dipping in a saturated aqueous solution of calcium hypochlorite. The sclerotia were plated at intervals from October 1940 through April 1941, and the desired fungus (*Corticium stevensii*) was isolated from each lot.

The plates were examined after 4 to 7 days incubation at about 24° C. in most cases; some were kept at room temperature. The colonies of *Corticium stevensii* were identified by microscopic examination. These forms have a characteristic type of branching and septation. Representative cultures were made from the different lots of sclerotia and compared with known cultures of *C. stevensii.*

During the course of the experiments more than 1500 sclerotia were plated, most of them from fig, but several hundred were from tung twigs. The percentages of sclerotia from which *Corticium* grew varied from about 35 to 94, but the numbers of sclerotia free of contamination were much lower. Some of the sclerotia had various faster growing fungi developing from them, which made it impossible to transfer the slower growing *C. stevensii.*

The results varied considerably with the different lots of sclerotia plated. One typical test is given here to indicate the results obtained. Sclerotia from tung and fig branches were subjected to 3 types of treatment. Each lot consisted of 50 sclerotia. Some lots were treated with the $HgCl_2$-alcohol solution for 2 minutes and rinsed in sterile water; others were dipped in the calcium hypochlorite solution after receiving the $HgCl_2$-alcohol treat-

[1] Wolf, F. A., and W. J. Bach. The thread blight disease caused by *Corticium koleroga* (Cooke) John, on citrus and pomaceous fruits. Phytopath. **17**: 689–709. 1927.

ment. Two lots of sclerotia were dipped in alcohol and then flamed. All lots were plated on water agar after being treated. The results obtained after 6 days' incubation at about 25° C. are given in table 1. Relatively

TABLE 1.—*Results obtained from plating thread-blight sclerotia from tung and fig branches after various treatments*

Host	Locality	Number of sclerotia plated	Treatment	Number giving growth of *Corticium stevensii*	Number from which *C. stevensii* could be obtained in pure culture
Tung	Bogalusa	50	[a]	22	7[d]
Tung	Bogalusa	50	[c]	22	2[d]
Fig	Union	50	[a]	46	40
Fig	Union	50	[c]	38	35
Fig	Union	50	[a]	41	24
Fig	Union	50	[a]	43	22
Fig	Union	50	[a]	33	19
Fig	Union	50	[b]	35	31

[a] = $HgCl_2$–50 per cent alcohol, 2 minutes.
[b] = $HgCl_2$–50 per cent alcohol, $CaOCl_2$.
[c] = Flamed in alcohol.
[d] = Much larger numbers of *C. stevensii* were obtained from later platings of sclerotia from tung.

large numbers of *Corticium stevensii* were obtained from most lots of sclerotia, the smallest numbers being found on those from tung twigs. The numbers of this slow-growing form that could be transferred varied from 2 to 40, but most of the lots had comparatively large numbers of colonies from which *C. stevensii* was successfully transferred.

This brief summary of studies carried on over a period of one year shows that pure cultures of *Corticium stevensii* can easily be obtained from sclerotia not more than 1 year old if they are soaked in a mixture of $HgCl_2$ 1–1000 in 50 per cent ethyl alcohol for 2 minutes, washed in sterile water, and plated on water agar. Other treatments also gave some good results.

LOUISIANA STATE UNIVERSITY,
UNIVERSITY, LA.

REPORT OF THE TWENTY-SIXTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAL PHYTO-PATHOLOGICAL SOCIETY

The 26th annual meeting of the Pacific Division of The American Phytopathological Society was held at the University of Utah, Salt Lake City, June 17–19, 1942, in conjunction with the annual meeting of the Pacific Division of the American Association for the Advancement of Science. Members were present from Arizona, California, Idaho, Utah, Wyoming, Washington, D. C., and Brazil. Twenty-two members attended the scientific sessions, 17 the dinner meeting, and 10 the field trip.

Sixteen volunteered papers were presented at 3 half-day sessions. At one half-day symposium on "Breeding for resistance to plant diseases," under the chairmanship of H. Loran Blood, and in cooperation with the Western Section of the American Society for Horticultural Science, 8 invitation papers were presented.

Officers of the society for the ensuing year are as follows: President, L. D. Leach, University of California, Davis; Vice-President, B. L. Richards, Utah Agricultural College, Logan; Secretary-Treasurer, C. E. Yarwood, University of California, Berkeley; and Councilor, Glenn A. Huber, Western Washington Experiment Station, Puyallup.

The next annual meeting is scheduled to convene in Corvallis, Oregon, in June, 1943.

Titles and abstracts of papers presented at the meeting follow.

C. E. YARWOOD,
Secretary-Treasurer.

Control of Crown Gall of Peach in the Nursery. ARK, P. A. Experimental data obtained in 1941 indicate that when peach pits are dusted with a slightly water-soluble chemical mixed with an inert insoluble material, such as Celite 500, there is a marked decrease in incidence of crown gall on the resulting peach stock. The following chemicals mixed at the rate of 15 g. per 450 g. of Celite 500 and applied to the thoroughly washed peach pits were not effective in preventing crown gall: calomel, copper carbonate, copper stearate, and thymol, showing 85.3, 94.3, 98.2, and 100 per cent disease, against 99.1 per cent in the check and 96.2 in Celite 500 dust. In contrast, Ceresan, mercuric cyanide, and mercuric iodide dusts showed only 3.8, 13.9, and 4.3 per cent disease. Acidification of the soil with agricultural sulphur, reducing the original pH of 7–8.5 to pH 5–5.5, caused a decrease in the amount of crown gall from 20 to 30 per cent. An application of 2000 lb. of sulphur per acre caused yellowing and stunting of the plants, while applications of 500 and 1000 lb. showed no injurious effects upon the peach stock.

Root Scab of Carrot Caused by Phytomonas carotae. ARK, P. A. and M. W. GARDNER. A scab of carrot roots, serious in California, and probably identical with one described by Ramsey and Wiant in 1941, results from direct root infection by *Phytomonas carotae*, cause of the bacterial blight of the leaves and umbels, described by Kendrick in 1934. Root infection abounds in irrigated fields cropped repeatedly to carrots. Very young lesions are small, rather inconspicuous, brown or maroon spots, often numerous. Early infection may result in sharply sunken constrictions of the root or large, rough, sunken cankers. Later infection results in laterally elongated, brown or black, rough, scabby lesions, often protruding because of the tremendous masses of the bacteria that ooze out and embed particles of soil. Particularly objectionable are the occasional fairly normal-appearing roots with healed-over internal pockets of blackened, infected tissue. Secondary fungi may invade infected roots. The bacteria may persist in the soil in the field as long as 6 months. Diseased roots culled out at harvest should not be left in the field. To prevent the introduction of the pathogen into noninfested soil, the seed may be treated for 10 minutes in a water bath at 50–52° C.

Longevity of Curly-top Virus in Dried Tissue of Sugar Beet. BENNETT, C. W. In July, 1934, small sugar-beet plants affected by curly top were dried at room temperature, divided into 4 lots, and each lot stored in a separate container under laboratory conditions. Lot 1 was exposed to the humidity of laboratory air throughout the test period. Lot 2 was placed over calcium chloride in an air-tight container. Lot 3 was placed over calcium chloride in an air-tight container and the air was replaced by hydrogen for the first year; during the remaining period of test, air was admitted. Lot 4 was stored in an air-tight container and the air was replaced by hydrogen for the first year; during the remaining period of test, air was admitted. Tests of active virus content were made at the beginning of the experiment and annually thereafter, except in 1940, for 8 years. Twenty seedling beets were inoculated from each lot in each test. The

plants infected in tests of successive years of test from each lot were as follows: Lot 1; 3, 3, 11, 0, 0, 0, 0, 0. Lot 2; 2, 2, 8, 3, 7, 0, 2, 0. Lot 3; 5, 1, 18, 1, 2, 2, 3, 2. Lot 4; 3, 0, 12, 0, 0, 0, 0, 0. These results show that when the virus is kept in a thoroughly dry condition it remains active for a period of at least 8 years.

Statistical Studies of Distribution of Psorosis Cases in Citrus Orchards. BITANCOURT, A. A. and H. S. FAWCETT. The majority of the cases of psorosis are apparently due to the utilization of buds from diseased parent trees and the different operations from the collection of bud sticks to the final planting in the orchard usually afford ample opportunities for a thorough randomization. However the comparison of the mean number of cases of psorosis in the 4 trees adjacent to diseased trees and adjacent to healthy trees has shown highly significant differences in 7 orchards, a significant difference in 1 and no significance in 3 orchards. In those 11 cases there were more diseased trees adjacent to diseased trees than to healthy ones. In an orchard showing very low percentage of psorosis, a nonsignificant difference was found in favor of the healthy trees. The same analysis made independently for the 2 adjacent trees along rows and the 2 adjacent ones along arrays has shown significant differences in both directions in 5 orchards, along rows only in 1 and along arrays only in 2. Systematic arrangement of diseased trees along rows or arrays at the time of planting is, therefore, indicated in the last 3 cases, while transmission from diseased to healthy trees is the most probable explanation for the 5 others.

Coryneum Blight of Stone Fruits. BLODGETT, EARLE C. Although present for many years and generally serious in northern Idaho, Coryneum blight of stone fruits has increased in recent years and become serious in the drier (southern) part of the State. The disease, usually confined to peach and apricot has occurred in extreme severity in commercial sweet and sour cherry orchards, and in smaller plantings of Italian and Agen (French prune). Inoculation studies using conidia of *Coryneum beijerinckii* from peach apricot, pin cherry [*Prunus emarginata* (Dougl.) Walp.] Cherry, Italian prune, and almond produced abundant leaf spots and twig lesions on potted trees in the greenhouse of Slappey Peach, Moorpark Apricot, Bing and Montmorency Cherry, Italian prune, Ne Plus Ultra Almond, and on Stanwick Nectarin (in the case of isolates from pin cherry, prune and almond). Some lesions appeared within 48 hours after inoculation. The tests showed that under the conditions encountered, the largest average number of spots per leaf, about 245, occurred with the isolates from peach and Royal Ann Cherry on Bing Cherry. The isolate from pin cherry produced the most severe spotting, 162, on prunes. Apparently *Coryneum beijerinckii* isolated from various hosts is pathogenic on the commonly cultivated stone fruits.

Effect of Temperature on the Epidemiology of Sugar-beet Downy Mildew. CARSNER, EUBANKS, CHARLES PRICE, and GLENN E. GILLESPIE. Downy mildew often develops extensively on sugar beets grown for sugar in the coastal areas of California and on sugar beets grown for seed in the Willamette Valley of Oregon and along Puget Sound in Washington. Temperature appears to be the most important factor influencing the epidemiology. Germination of the spores and infection will occur at relatively low temperatures. Sporulation requires slightly higher temperatures. All development of the fungus stops at about 70° F. and infected plants that have not been too severely injured begin to recover.

Shock Effect of Certain Viruses of the Mosaic Group on Peach. COCHRAN, L. C. Observations and surveys in mosaic-affected peach orchards have substantiated the suggestions of growers and inspectors that severely retarded trees are initial cases of the disease. Less severely affected trees of tolerant varieties often show symptoms only during the initial year of the disease. Transmission annually for 3 years from individual severely and mildly affected orchard trees has shown symptoms to be much more acute in the respective test trees the first year after inoculation. Subsequent transmission from such test trees, after partial recovery, gave acute symptoms approximating those of the first year of infection in the first set. The ring-spot virus of stone fruits produces acute symptoms the initial year of the disease in peach but only rarely shows any symptoms the second year, in which cases the tree was not universally invaded the first year. Subsequent inoculations from symptomless carrier trees result in acute symptoms. In other hosts where the ring-spot virus causes symptoms annually, symptoms are more intense during the first year of the disease. Similar results have been obtained with two undescribed viruses causing mottling on peach. This phenomenon is regarded as shock effect correlated with the first stage of the disease.

Limitations in the Control of the Virus Diseases of Peach Through Plant Breeding. COCHRAN, L. C. Prior to 1931 all viruses known to infect peach affected all horticul-

tural varieties similarly, moreover, some infected other stone fruits that could be hybridized with peach. This afforded no starting point for the plant breeder and left no alternate to control through infected tree removal. Soon after the discovery of the peach-mosaic disease it was noted that certain peach varieties could be affected with little damage. While this appeared encouraging, it was soon found that such varieties were reservoirs from which the severe form of the virus could spread. Work with vigorous sport-like shoots on severely mosaic-affected trees and with immunizing trees with a mild form of the virus has shown promise; but both host and virus stability need further investigation. The occurrence of virus strains and variance in symptom intensity within the mosaic complex has further complicated the problem. The production of a desirable immune variety is remote, and would involve a long-time, intensive breeding program. Numerous recently discovered peach viruses have appeared in the literature. Some of these, as the X disease, appear to infect and damage all peach varieties similarly, and some are too cosmopolitan in host range to offer much hope to plant breeders.

Breeding Beans for Resistance to Powdery Mildew and Rust. DUNDAS, B. By testing detached leaflets in Petri dishes the reaction of individual plants of segregating populations to the various forms of mildew and rust can be determined. It is thus possible to determine the number of relationships of the genetic factors giving resistance to these several forms. Pinto, other field beans, and some garden varieties carry a main dominant factor for resistance to 12 of the 14 forms of mildew isolated. This factor is being incorporated into new garden beans now under development. There is also a dominant factor for semi-resistance, and one causing susceptibility during 5–7 days after emergence. A number of factors for resistance to rust have been found in various garden beans. The proper combination of certain of these factors should give resistance to all 20 known forms of rust. A combination of the factors for resistance in Golden Gate Wax and Brown Kentucky Wonder 928 has been used in breeding for resistance to 4 forms of rust. Recently several new varieties have been released that are resistant to some forms of rust. Other promising lines carrying resistance to more forms of rust, mildew, and bean virus No. 1 should be forthcoming.

Perithecia of Bean Powdery Mildew. DUNDAS, B. In the fall of 1941, immature perithecia were independently found by Middleton and Yarwood on Lima beans infected with powdery mildew, in Southern California. The writer subsequently established the fact that conidia from the leaves containing perithecia always produced infection of form 1, the form most commonly found on Lima beans. Assuming these perithecia are from the same mildew which produced the conidia, the appendages showed them to belong to the genus *Microsphaera* and not to *Erysiphe,* as has been previously supposed.

Wood Alteration in Psorosis of Citrus in Relation to Tree Decline. FAWCETT, H. S., A. A. BITANCOURT, and J. M. WALLACE. In psorosis A and B, (1) the primary lesions in the wood consist of gum in and between part of the vessels, (2) secondary lesions in the wood result in irregular discoloration and necrosis. The latter usually develop after the branch or trunk is girdled by the primary lesions of the bark and wood. Suction experiments in drawing water or aqueous stains (as 0.5 per cent acid fuchsin or sufranin) through the wood indicates that the presence of the primary lesion has little effect on water passage, but that the secondary lesions, as well as a region contiguous to the visibly affected part are impervious to water passage. These regions are coextensive with those in which the I-KI test shows absence of starch. Experiments in drawing air through wood of branches showed that larger amounts of air passed through bark with psorosis lesions than through normal bark and that in normal bark air passed slowly only through the lenticels. The lack of water passage caused by formation of the secondary wood lesions appears to be the principal factor in tree decline. The alterations and discoloration of the central wood layers probably are explained by their starvation due to blocking the passage of food inward by gum in the primary wood lesions nearer the surface.

Progress in Breeding Sugar Beets for Curly-top Resistance. OWEN, F. V., ALBERT M. MURPHY and BION TOLMAN. Curly-top-resistant varieties of sugar beet have now definitely revived the sugar-beet industry in the Western States. In many tests high yields have been obtained with the resistant varieties where the susceptible European varieties failed completely. U. S. No. 1, grown commercially on a limited scale in 1934 and extensively in 1935, was the first curly-top-resistant variety released by the United States Department of Agriculture. Varieties U. S. 34, U. S. 33, and U. S. 12 soon replaced U. S. 1, but of these the high sugar-producing variety U. S. 33 is the only one that will be continued in commercial use. U. S. 22, a new variety with a higher degree of curly-top resistance, is now available to commercial growers. If given good cul-

tural care, the U. S. 22 beets make good growth in spite of curly-top infection; but, if neglected and allowed to suffer for irrigation water, some curly-top damage may result. The advantage of having still higher curly-top resistance is obvious, and new improved selections are being propagated as rapidly as possible. The variety U. S. 1 and all other curly-top-resistant varieties so far produced have been highly self-sterile, a feature that has made mass selection the principal means of improvement. However, many curly-top-resistant self-fertile lines are now available to facilitate inbreeding work.

The Big Vein Disease of Lettuce in Relation to Soil Moisture. PRYOR, DEAN E. An investigation of soil moisture in relation to the big-vein disease of lettuce was made on two different infective soils, one from near Salinas, California, and the other from the Imperial Valley, California. The number of big-vein plants appearing on highly infective soil increased with the increasing moisture. A considerable number of diseased plants was present, even at moisture levels below the optimum for growth of lettuce, and a few plants had big vein when supplied with only enough water to keep them alive. The greatest disease incidence occurred in the treatments producing the largest plants. Plant weight increased with each increment of moisture used. The more vigorously growing individuals in each series seemed to be most readily affected with big vein, but the earlier a plant showed the disease the smaller was its final weight. In the Salinas soil, diseased plants seemed to be larger than healthy plants in the same pot, while the reverse was true in Imperial Valley soil. The difference was attributed to a longer growing period for plants in the Imperial Valley soil.

Morphological Similarity in Culture Between Torulopsis pulcherrima and Taphrina deformans. ROBERTS, CATHERINE. Three- to five-month-old cultures of *Torulopsis pulcherrima* and *Taphrina deformans* on potato dextrose and vegetable (beet-cucumber-carrot-potato) agar exhibited decided similarities in cellular morphology. In 2 out of 4 isolates of *Torulopsis pulcherrima* globose, thick-walled cells containing single fat globules and with attached remnants of cell walls were observed. Other cells possessed single bud-like protrusions containing 1 to 2 spores, thus partially confirming observations on sporulation made by Windisch in 1940, who interpreted the globose cell as an ascus mother cell, the protrusion as an ascus, and the attached wall remnant as the remains of an ascus following ascospore discharge. In *Taphrina deformans* similar globose, thick-walled cells containing one to many fat globules were observed, together with attached wall remnants. Bud-like protrusions also were present, some of which contained 1 to 3 ellipsoidal bodies believed to be ascospores, although their discharge and germination have not been observed. These observations, together with the similarity evident in macroscopic cultural behavior, including dissociation into pigmented and non-pigmented areas, suggest that *Taphrina deformans* and *Torulopsis pulcherrima*, at least those isolates studied, are more closely related than is indicated by their positions in the present systems of fungus classification.

Attempts at Mass Infection of California Citrus Red Scale with Bacteria. SOKOLOFF, V. P. and L. J. KLOTZ. Approximately 5×10^{12} spores of the red scale bacillus (Phytopath. 32: 187–198) were sprayed and dusted over lemon trees in 2 field trials conducted in September and December, 1941. A demonstrable increase in mortality of the scale was noted on leaves, green bark, and fruits of the sprayed-dusted trees. Density of the red scale population decreased significantly on the infected trees, parallel with the decreased percentage survival. In the December experiment a premature mortality developed among the scale throughout the 2-acre grove, on the 60th-70th day, whereupon differences between the controls and the infected trees had disappeared. The microorganism used in the experiment and some other species of the genus *Bacillus* could be isolated invariably from the prematurely dead adults and gray adults in sections of the orchard far removed from the site of the experiments. Among other organisms associated with the premature death of the scale, there were some common, apparently non-pathogenic fungi and a species of *Actinomyces*.

The Effects of Some Natural Gases Upon Plants. SOLHEIM, W. G. and RALPH W. AMES. The effects of a natural gas from the Billy Creek field, source of the gas used in Sheridan, Wyoming, and one from the gas mains of Laramie, Wyo., were studied. (The gas from the Billy Creek field was supplied through the courtesy of the Northwest States Utilities Company of Sheridan, Wyo.) The composition of the Sheridan gas, according to an analysis made by the U. S. Bureau of Mines, is as follows: carbon dioxide 0.2 per cent; oxygen 0.2 per cent; methane 97.5 per cent; ethane 0.0 per cent and nitrogen 2.1 per cent. According to an analysis made by the Kansas City Testing Laboratory of Kansas City, Missouri, the Laramie gas has the following composition: methane 71.5 per cent; ethane 22.3 per cent; carbon dioxide 0.0 per cent; oxygen 0.2 per cent; olefines 0.0 per cent and nitrogen 6.0 per cent. Tomato, potato, sunflower, castor bean, and

geranium plants were in no way affected by concentrations of 4, 6, 12, 24, and 50 per cent for 24-, 48-, 72-, 96-, and 120-hour treatments. Cut carnations did not exhibit any symptoms of injury when treated with 1 and 2 per cent concentrations for 24, 48, 72, and 96 hours. When Fuchsia plants were exposed to gas concentrations of 4, 12, 24, and 50 per cent for 24, 48, 72, and 96 hours a slight browning and wilting of the petals was noted. The tests reported above were made in 1940. In 1941 further tests were made on tomatoes with gas from the Billy Creek field. These tests confirmed the original ones —no effects being observed from the gas.

Investigation of Storage Diseases of Carrots. VIRGIN, W. J. A considerable amount of carrot seed is produced in Idaho each year. The climatic conditions are such that it is necessary to store the roots through th winter and set them out in the spring, rather than leave them out in the field all winter. The grower either stores them in a pit or a potato cellar. Each year there is considerable loss in storage due to the organisms *Botrytis cinerea* and *Alternaria radicina.* At near-freezing temperature growth of these organisms is almost stopped. Consequently, the best storage is the one in which the temperature remains near freezing. It was found that pit storage was superior to cellar storage, since the temperature could be maintained near 0° C. and it did not fluctuate so much as did the cellar temperature. At temperatures above 5° C. carrot roots in storage soon produced new leaf growth. These new leaves were very susceptible to infection by *B. cinerea,* and, from the leaves, the organism soon invaded the crown and completely destroyed it, rendering the carrot useless for seed production. The other important point of entry for *B. cinerea* was the fine tip end of the tap root.

Function of Lime and Host Leaves in the Fungicidal Action of Bordeaux Mixture. YARWOOD, C. E. To give 95 per cent inhibition of germination of bean-rust urediospores on glass surfaces required a dried deposit of 3.2 mg. per sq. dm. of bluestone or 450 mg. of equal lime-Bordeaux mixture. To give 95 per cent reduction in number of uredial pustules of bean rust (= 95 per cent control) in greenhouse tests under conditions of heavy artificial inoculations required 4.5 mg. bluestone but only 0.44 mg. Bordeaux mixture per sq. dm. of lower leaf surface. Therefore, to give equivalent control, 1000 times as much Bordeaux was required on glass as on leaves, more bluestone was required on leaves than on glass, bluestone was more toxic than Bordeaux on glass. Bordeaux, however, was far more fungicidal than bluestone, on leaves. The copper in bluestone spray was rapidly absorbed by bean leaves with consequent reduction in control, but the copper in Bordeaux was absorbed to a much smaller extent. These results indicate important unreported functions of lime in Bordeaux mixture; but the above principles have not as yet been confirmed with cucumber downy mildew.

Fungicide Treatment of String Supports for Control of Hop Downy Mildew. YARWOOD, C. E. In California the principal damage from hop downy mildew is due to infection of the nodes and growing points of the trained vines. This infection usually occurs during rainy periods. Because the terminal growing points and nodes are close to the strings on which the vine is trained, and since Magie has shown (Phytopath. **30:** 16, 1940) that the drip from Bordeaux-sprayed hops protected non-sprayed growth, it was thought that if the strings were impregnated with a suitable fungicide, enough of the fungicide might be scattered by splashing and dripping rain to kill the spores at the nodes and growing points. String treatments with various copper, sulphur, and organic materials were tested in 6 hop yards in 1942. The natural drip during rain from cotton strings treated with low lime-Bordeaux (7.5 per cent bluestone plus 2.5 per cent lime) when diluted to 1/20 its field strength prevented germination of the sporangia of *Pseudoperonospora humuli* in watch glasses. Vines growing on strings treated with the low lime-Bordeaux had 80 per cent fewer leaf infections, 50 per cent fewer nodal infections, and 78 per cent fewer infected basal shoots than vines on untreated strings in one hop yard.

THE WAR EMERGENCY COMMITTEE

of

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The purpose of the War Emergency Committee is to provide for coordinated effort in research, experimentation, and extension work designed to control destructive diseases of plants. To accomplish this aim there is a general committee, comprising a number of members at large and the chairmen of regional groups that perform the same function in certain natural geographical divisions as the general committee does for the country as a whole.

Past experience indicates very clearly that it is imperative in war time to maintain an efficient plant quarantine service. New diseases and pests are very likely to be introduced and distributed because of the exchange of plant propagative materials between certain countries and within the United States. The expansion of acreage of certain crops and the consequent interchange of seed and other propagative parts of plants intensify the problem. Indeed, some new diseases already have made their appearance during this war. Furthermore, there is always the likelihood that the increase in prevalence of new parasitic strains of pathogens already present may develop on hitherto resistant varieties of crop plants; consequently it is of the utmost importance to maintain adequate plant disease survey services in order to provide for the early detection, extermination, and control of new diseases or of unusually virulent strains of old disease organisms.

Two of the best methods for preventing the indiscriminate distribution of plant pathogens are seed certification and seed treatment. For this reason, provision has been made in the organization of subcommittees for cooperative effort in improving seed certification and for obtaining new information and disseminating old information regarding fungicides used to treat seeds and other propagative parts of plants.

Because of priorities there is a shortage of certain commonly used fungicides, and the need therefore develops for substitute fungicides. A subcommittee is working hard and effectively on this problem and, in cooperation with fungicide manufacturers and with governmental agencies, is attempting to develop substitute fungicides and to determine the most economical ways of using those now available.

It is of course of the utmost importance to provide for the proper dissemination of essential information regarding plant diseases, and a subcommittee on extension activities is therefore attempting to codify important control measures and to assist in obtaining provision for proper dissemination of the information.

During World War I, a War Emergency Board of American Plant Pathologists under the chairmanship of Professor H. H. Whetzel made significant contributions to insuring the nation's food supply through the control of plant diseases. The situation with respect to foodstuffs has not yet become acute in this war, but the situation with respect to some essential plant materials already is acute, and the control of plant diseases and insect pests is therefore of paramount importance. The American Phytopathological Society, through its War Emergency Committee, is again attempting to pool the services of plant pathologists insofar as possible, in order to alleviate present situations with respect to plant diseases and to safeguard the future. The membership at a summer meeting in Toledo, June 25 and 26, discussed war problems extensively and attempted to provide insofar as possible for the solution of immediately pressing problems. It was the consensus of opinion that basic research designed to elucidate principles on which control measures must be based is even more important in war time than in peace time, in order to avoid mistakes in the present and to lay a solid foundation for future procedures.

The personnel of the Committee and its Subcommittees follows:

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